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Gaseous Exchange and Wetland Plant Response to Soil Redox Conditions.

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Gaseous exchange and wetland plant response to soil redox conditions

Kludze, Hillarius Kodzo, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1994

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Ann Arbor, MI 48106

GASEOUS EXCHANGE AND WETLAND PLANT RESPONSE TO SOIL REDOX CONDITIONS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Agronomy

by
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May, 1994

DEDICATION

To Marion Boulden, Augustine, Desmond and Alphonse Kludze

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ABSTRACT

Laboratory studies pertaining to effects of soil redox conditions on gas exchange and plant responses were undertaken. A colorimetric method for assaying O_2 concentration in the root rhizosphere was used to quantify radial O_2 loss from plant roots.

The oxidizing power of seven rice cultivars, grown under aerobic and hypoxic conditions, was measured colorimetrically with titanium-citrate solution. Oxygen release rate was calculated by extrapolation of measured absorbance to standard curves after placing plant roots in the solution for 6 h. Since Ti^{3+} ions readily react with O_2 as it is released from roots, the technique provides a sensitive measurement of dissolved O_2. Variations in O_2 release rates existed among cultivars and between aeration status. Values ranged from 10.0 to 18.6 μmol O_2 plant^{-1} d^{-1} in the aerobic treatments, and from 10.4 to 33.2 μmol plant^{-1} d^{-1} in the hypoxic treatments. Oxygen leakage from hypoxic treatments was highly correlated to root porosity in some cultivars, which may explain their success in low-land cultivation in Louisiana.

There were interactive effects of cultivar type and straw application rate on rice growth and gaseous transport. Results indicated that inhibition of CH_4 emissions at high straw dosage was not due to inhibition of methanogenesis but due to plant-related factors that influence net CH_4 emissions.

Soil reduction intensity affected porosity and gas transport in the two test plants, *Oryza sativa* and *Spartina patens*. Plants were grown in Crowley soil (Typic Albaqualf) and Mississippi alluvial soil (Typic Fluvaquent), respectively, under controlled redox intensity levels, +200, -200 and -300 mV. Results demonstrated that soil Eh influences net CH_4 emissions by (1) directly determining the amount and rate of CH_4 production in the soil, and (2) initiating morphological and physiological changes in aquatic plants that affect gas exchange between the soil and the atmosphere.

Plant growth and CO_2 fixation responded differently to soil redox intensity and capacity, while other parameters measured did not respond to redox capacity. Soil redox intensity and capacity must therefore be considered for properly evaluating physiological responses of wetland plants to reducing conditions in the root medium.

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

In recent years, there has been an increasing interest in the investigation of plant physiological changes and the emission of the so-called biogenic greenhouse gases (CO_2, CH_4 and N_2O) in response to wetland conditions. Wetlands are characterized by the saturation of their soils with water, resulting in reducing conditions which may be quantified in intensity and capacity terms. The intensity factor determines the relative ease of the reduction, while the capacity factor denotes the amount of the redox system undergoing reduction (Reddy *et al.*, 1986; DeLaune and Pezeshki, 1991). Reducing conditions occur as a result of depletion of O_2, followed by a sequential reduction of the inorganic redox system or alternate electron acceptors (NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-} and finally CO_2) as predicted by thermodynamics considerations (Stumm and Morgan 1981; Ponnamperuma, 1984). Such reducing conditions have two major consequences on the growth and adaptation of plant species: the over-abundance of water for essential physiological functions of the plant and the curtailment of soil oxygen supply, which affects root and microbial respiration and also initiates a chain of microbial processes, including CH_4 gas production. The addition of exogenous carbon substrates such as straw, speeds up the microbial processes which further enhance O_2 depletion and CH_4 production.

Survival of wetland plants under reducing conditions is partly dependent on the adaptational ability of these plants to conduct O_2 from the atmosphere to the roots and rhizosphere via the shoot (Armstrong, 1979; Burdick and Mendelssohn, 1990). In the absence of this internal gas transport, flood tolerance must depend on a strong capacity for anaerobic generation of energy (Saglio *et al.*, 1988; Burdick and Mendelssohn, 1990). Root air-space (porosity) which is an indication of aerenchyma tissue formation, is considered to be an essential adaptation of wetland plants to survive reduced conditions (Schat, 1984; Drew *et al.,* 1985). The root air-space provides a channel for the transport of gases to and from the root medium. Thus, a well ventilated root system would provide O_2 to roots and the rhizosphere whiles at the same time provide a pathway for the escape of CH_4 gas to the atmosphere. However, there is a paucity of information on plant root air-space formation and its concomitant release of O_2 into the rhizosphere, in response to well defined levels of soil reduction intensity and reduction capacity.

In recent years there has been a great public concern about global warming resulting from atmospheric increases in the greenhouse gases. Methane receives more attention as a greenhouse gas because of its comparatively high increases in annual emissions with its consequential impact on the earth's climate and the chemistry of the atmosphere. Methane is produced by strictly anaerobic methanogenic bacteria that are most commonly found in waterlogged anoxic (highly reduced) soils such as rice paddies and natural wetlands (Schutz *et al.*, 1991). The net total emission of CH_4 into the atmosphere is the total production reduced by the oxidation in the methanogenic environment. Although it is well acknowledged that wetland plants may be actively involved in the processes of CH_4 production, oxidation and its final emission, there is scarcity of information on the integrated effect of soil anaerobiosis (both intensity and capacity) on CH_4 production and on plant morphology, both of which can interactively modify net CH_4 emissions.

Considering the importance of O_2 in plant survival and carbon cycling in wetland ecological systems, there is the need to have good estimates of the amount of O_2 conducted from the atmosphere into the rhizosphere of wetland species, and relate such quantified estimates to plant performance and CH_4 emission. Methods of soil solution O_2 assay include the use of platinum wire electrodes (Boon and Sorrell, 1991), and other techniques such as gas chromatography, mass-spectrometry and colorimetry. Although these techniques have been in use for years, some of them have been criticized for their lack of accuracy. For example although the polarographic method is commonly used, it suffers from the "boundary layer effect" which may underestimate measurements. In the case of colorimetric methods, their accuracy and/or slow rate of reaction of reagents in the presence of O_2 have been questionable. Recently, DeLaune *et al.* (1990) used the oxidized (Ti^{4+}) and reduced (Ti^{3+}) forms of titanium-citrate buffer solution to study the photosynthetic activity of *Zea mays* and *Spartina patens*, and postulated that Ti^{3+}-citrate solution could be used to quantify O_2 transport through the aerenchyma to the rhizosphere.

The objectives of this research were to:

(1) test a colorimetric method of assaying radial oxygen loss from plant roots in solution. Titanium-citrate buffer, whose color change is proportional to O_2 concentration in a medium was used for the test. The technique is based on the principle that any O_2 released to the rhizosphere is irreversibly consumed by the buffer solution, indicated by change in color.

(2) evaluate the interactive effects of cultivar type and straw application rate on rice growth and gas transport.

(3) determine the effects of soil reduction intensity on root porosity, O_2 release rate, plant growth and the rates of CH_4 production, emission and oxidation on two wetland species: rice (*Oryza sativa)* and wiregrass (*Spartina patens*).

(4) differentiate between plant responses to soil reduction intensity and reduction capacity.

The first objective was accomplished by quantifying O_2 loss from whole root system of seven rice cultivars with the titanium-citrate buffer solution. The effect of short-term hypoxia on root porosity was also evaluated. The experimental procedure, results and conclusions of this study are presented in Chapter 1. The interactive effect of cultivar and exogenous organic matter (straw) application rates on plant growth, root porosity and gas transport in rice was evaluated in Chapter 2. Effects of soil reduction intensity on plant growth, root porosity, CH_4 production, emission and oxidation, and radial oxygen loss under specific controlled redox levels in rice and wiregrass are presented in Chapters 3 and 4, respectively. The study on the differences between plant responses to soil reduction intensity and capacity in the two test plants are reported in Chapter 5. All experiments were conducted under laboratory conditions.

LITERATURE REVIEW

Soil Reduction processes during flooding

Theory of oxidation-reduction potential (Eh)

Roots and soil organisms obtain their energy from the oxidation of organic substances in a series of enzyme-catalyzed reactions involving a combination of proton and electron transfer (Baas Becking *et al.*, 1960) from respirable substrates along a series of organic acceptor molecules. In aerobic soils and sediments, the terminal electron acceptor is oxygen. Upon flooding, oxygen becomes depleted in the soil or sediment and alternate electron acceptors assume the function of oxygen. The source of electrons in the soil is organic carbon while their acceptors are oxidized inorganic compounds.

One of the most important electrochemical properties of the soil that is affected by flooding is the oxidation-reduction potential, Eh. Oxidation-reduction or redox potential is a measure of the electrochemical potential or electron availability in chemical and biological systems (Gambrell and Patrick, 1978). Redox reactions can be represented by the following reduction equation:

$$Ox + mH^+ + ne \text{-----}> Red \quad (1)$$

where Ox is the oxidized component or electron donor, m and n are the number of hydrogen ions (protons) and electrons involved in the reaction, respectively, and Red is the reduced component or electron acceptor. The driving force of any chemical reaction is a tendency to decrease free energy of the system until, at equilibrium, the sum of the free energy of the products is equal to that of the remaining substrates. Thus, the reduction reaction can be defined quantitatively through the change in Gibbs free energy (ΔG) given as:

$$\Delta G = \Delta G_o + RT\ \mathrm{In} \frac{[Red]}{[Ox]} - mRT\ \mathrm{In}\ [H^+] \qquad (2)$$

where ΔGo is the standard free energy change, R is the universal gas constant, T is absolute temperature, and [Ox] and [Red] are the activities of the oxidized and reduced forms, respectively. The Nernst equation expresses the reaction in terms of electrochemical energy (volts or millivolts) , using the relationship: $\Delta G = -nEF$, which is generally written as:

$$Eh = E_o + RT\ \mathrm{In} \frac{[Ox]}{[Red]} + \frac{mRT}{nF}\ \mathrm{In}\ [H^+] \qquad (3)$$

where Eh is the electrode potential or the electromotive force of the reaction in volts measured with respect to the standard hydrogen electrode, $[H^+]$ is hydrogen ion activity, Eo is the standard half-cell potential or the voltage when $[Ox]=[Red]$ and $[H^+]=1\ \mathrm{mol\ dm^{-3}}$, and F is the Faraday's constant (9.65 x 104 C $\mathrm{mol^{-1}}$ electron). At 25 °C, Eh is simplified by the equation:

$$Eh_{(V)} = Eo + \frac{0.059}{n} \log \frac{[Ox]}{[Red]} \qquad (4)$$

This relationship can also be described by using the *pe* value which is the negative logarithm of electron activity: $pe = -\log [e]$. At 25 °C, $pe = \frac{Eh}{0.059}$

Thus, description of a redox system in terms of the redox potential (Eh) and pe are equivalent to each other due to the above relationship.

Soil redox intensity and capacity factors

The reduction of the inorganic redox system in the soil subsequent to flooding can be described in both intensity and capacity terms. The intensity factor determines the relative ease of the reduction, whereas the capacity factor denotes the amount of the redox system undergoing reduction, such as oxygen consumption at the root surface (DeLaune and Pezeshki,1991). The intensity factor can be represented by the free energy of the reduction, or more commonly by the equivalent electromotive force (EMF) of the reactions. The oxidation-reduction potential (Eh) is ordinarily used to denote the intensity of reduction. In soil and sediment-water systems, many redox couples are usually present and most are not chemically reactive with others. Electrodes used to measure redox potential are not specific for a single redox couple. Thus, the electrode (usually platinum), responds to the electrochemical potentials of all redox couples present, so that the resulting measurement represents a weighted average of the potentials contributed by each of the redox couples present in the system (Bohn, 1968). This weighted average of the redox potential is ordinarily used to denote the intensity of reduction. Patrick and Mahapatra (1968) suggested four general ranges of redox potential usually encountered in oxidized and reduced soils; at pH 7, oxidized soils are characterized by a redox potential of $>+400$mV, moderately reduced soils from +400 to +200mV, reduced soils from +200 to -100mV, and highly reduced soils from -100 to -300mV. At Eh $<+350$ mV, there is absence of O_2 (DeLaune and Pezeshki, 1991).

Although knowledge of the initial redox potential at which the organic redox systems become unstable and are reduced provides invaluable information, it provides no indication of the total capacity of the system to accept electrons and thereby support respiration. For this reason, it is essential that an understanding of the capacity factor in redox reactions in the soil be obtained. The capacity factor is equivalent to the total amount of electrons accepted by the soil oxidants (electron acceptors) in microbial respiratory activity; it may also refer to the total amount of labile carbon compounds that are utilized during microbial activity which is a reflection of oxygen demand. Thus, the capacity factor of the various redox systems will vary from one soil to another. Oxygen consumption equivalent best describes the capacity factor. The amount of O_2 at the time of flooding in a well-drained soil is generally very small and consists of O_2 in the trapped air spaces plus that dissolved in the water occupying the pore space. The amount of NO_3^- present at flooding is likely to be more variable than O_2 but is usually only a few parts per million (ppm). Reducible Mn oxides are present in much more greater concentrations, with most soils having less than 100 ppm reducible Mn, while others have over ten times as much. Most soils have much greater amounts of reducible Fe compounds than of any other inorganic redox component. Sulfate is also a variable oxidant and occurs mostly in coastal salt marshes.

Sequential reduction

The most important change in the soil as a result of waterlogging is the conversion of the root zone of the soil from an aerobic environment to an hypoxic or anoxic condition where O_2 is limiting or completely absent. Subsequent to complete exhuastion of O_2 in waterlogged/flooded soils and sediments, a sequential reduction of the inorganic redox system ensues as predicted by thermodynamic considerations (Turner and Patrick, 1968; Stumm and Morgan, 1981; Ponnamperuma, 1984). The affinity for electrons by these components depends upon their electron bonding energies (Patrick and Reddy, 1978). In the presence of readily available energy source, NO_3^-, Mn^{4+} and Fe^{3+} are utilized by facultative anaerobes and reduced to N_2/N_2O, Mn^{2+} and Fe^{2+}, respectively. Under intensely reduced conditions, SO_4^{2-} and finally CO_2 are utilized by obligate anaerobes to produce H_2S and CH_4, respectively.

In addition, organic substrates that are not decomposed completely to CO_2 or CH_4 (DeLaune *et al.*, 1976; Ponnamperuma, 1972) under reducing conditions, form incompletely oxidized intermediates and end-products through hydrolysis and fermentation processes; these include lactic acid, ethanol acetaldehyde, volatile hydrocarbons and aliphatic acids such as formic, acetic or butyric acids (DeLaune and Pezeshki, 1991) and also ethylene (Smith and Jackson, 1974).

Methane production (methanogenesis)

The biological formation of CH_4 (methanogenesis), is an important process that takes place in all anaerobic environments in which organic matter undergoes decomposition. Such environmemts include lakes, wetlands, paddy fields, the digestive tracts of ruminants and termites. Methanogens are important because they represent the terminal steps in the food chain of these anaerobic environments where organic carbon is ultimately released as CH_4.

Although microbial methanogenesis has been known since 1876, elucidation of the biochemistry of the process dates from the 1930s (Barker, 1956; Oremland, 1988). Methane results from the metabolizing activity of a highly specific bacterial group called the archaebacteria. These strictly anaerobic bacteria convert the fermentation products formed by other microorganisms into CH_4. Some of the possible reactions with reference to the type of methanogens involved, include the following (adapted from Papen and Rennenberg):

(a) H_2 reduction of CO_2 by obligate chemoauthotrophic methanogens:

$$CO_2 + 4H_2 \text{-----}> CH_4 + 2H_2O$$

(b) use of methanoic acid (HCOOH) or CO by several strains of methanogens:

$$4HCOOH \text{-----}> CH_4 + 3CO_2 + 2H_20.$$

$$4CO + 2H_2O \text{-----}> CH_4 + 3CO_2.$$

(c) production of CH_4 by methylotrophic methanogens which use methyl-group containing substrates such as methano, acetate and trimethylamine (Papen and

Rennenberg, 1990):

$$4CH_3OH\text{------}>3CH_4 + CO_2 + 2H_2O$$

$$CH_3COOH\text{-----}>CH_4 + CO_2$$

$$4(CH_3)_3\text{-}N + 6H_2O\text{----}>9CH_4 + 3CO_2 + 4NH_3.$$

In nature, CH_4 is mainly produced from H_2-CO_2 and from CH_3COOH (Schutz *et al.*, 1989). In the rice rhizosphere for example, CH_4 production was considered to proceed in two steps (Kimura *et al.*, 1984): in the first step, H_2 is produced by microorganisms and/or inside the roots; in the second step, H_2 is transferred to the rhizosphere soil and converted to CH_4 by methanogenic bacteria living in the rhizosphere soil. However, Takai and Wada (1990) recognize the transmethylation of CH_3COOH as being the most important biochemical pathway of CH_4 formation in waterlogged paddy soils. Even under favorable conditions, methanogenesis could be hindered by competitive demand for CH_3COOH and H_2 by sulfate reducing bacteria and CH_4-producing bacteria (Cappenberg, 1975; Lovely and Klug, 1975; Westermann and Ahring, 1987). The consumption of H_2 by the methanogens is often important in maintaining low enough H_2 partial pressures to permit active growth of acetogenic bacteria that produce H_2, yet are inhibited by its accumulation. This phenomenon of "interspecies H_2 transfer" is important in many anaerobic ecosystems (Wollin and Miller, 1987).

Recently, Lindau *et al.* (1993) reviewed the factors affecting CH_4 production in flooded paddy soils and reported that methanogens can function in almost any anaerobic ecosystem and can withstand salinity, pH and temperature extremes but cannot function in the presence of O_2 and other oxidized inorganic compounds (Van Breemen and Feijtel, 1990). Factors influencing soil CH_4 production include redox potential (Eh), pH, temperature, organic matter content, soil type and the addition of chemicals or fertilizers (USEPA, 1990). Since methanogenic bacteria are very strict anaerobes and intolerant of O_2 exposure for growth, they require low redox potential

(Eh =-200 mV and below) associated with rapid decomposition of organic matter. High concentrations of oxidized inorganic species would therefore delay or inhibit methanogenesis. Field studies conducted have shown that increasing CH_4 emissions are directly related to Eh decreases in paddy soils (Cicerone *et al.*, 1983; Yagi and Minami, 1990; Lindau *et al.*, 1991) and in natural wetlands (Kyuma, 1985). Like other biochemical reactions, temperature is an important regulator of methanogenesis (Schutz *et al.*, 1990b). In nature, methanogens may be mesophilic or thermophilic. Dunfield *et al.* (1993) reported that the optimum temperature for methanogenesis is about 25 °C. There is very little or no methanogenic activity at temperatures of 0 to 10 °C (Dunfield *et al.*, 1993). Most methanogenic bacteria grow in the pH range of 6 to 8 and the lowest pH for growth of any species is 5.6 (Garcia, 1990). In flood rice systems, methanogenesis is favored by a near neutral pH (7), but is influenced by the soil type (Bouwman, 1991; Wang *et al.*, 1993; Yagi and Minami, 1990; Sass *et al.*, 1990). For example, in a study of CH_4 emissions from Japanese paddy soils, Yagi and Minami (1990) made the following classifications: peaty soil> alluvial soil> Andosol, while Wang *et al.* (1993) reported that soils with relatively the same amounts of organic carbon differed in CH_4 emissions because of differences in biological reducible Fe and Mn. Soil texture (Parasher *et al.*, 1991), as much as the amount and type of organic matter (Yagi and Minami, 1990; USEPA, 1991), also play significant roles in CH_4 production.

Methane oxidation/consumption (methanotrophy)

In the presence of O_2, some ecosystems that produce CH_4 can also function as sinks (Seiler *et al.*, 1984; Steudler *et al.*, 1989; Schutz *et al.*, 1990a). Bacteria that consume CH_4 for growth are known as methanotrophs and are a larger grouping of organisms termed methylotrophs. All methanotrophs isolated and studied to date are obligate aerobes since the enzyme responsible for the initial step in CH_4 oxidation is a monooxygenase enzyme that requires molecular O_2. Methanol, the product of the latter reaction is further successively oxidized via formaldehyde to formate and then to CO_2. The complete chain of reaction may be represented as follows (after Papen and Rennenberg, 1990):

CH_4 + NADH $+H^+$ $+O_2$---->CH_3OH + NADH + H^+ + H_2O

CH_3OH + methataxin (PQQ)------>CH_2O + $PQQH_2$

CH_2O + NAD^+ + H_2O-------->HCOOH + NADH + H^+

HCOOH + NAD^+-------->CO_2 + NADH + H^+

According to Papen and Rennenberg (1990), some aerobic chemoautotrophic NH_4^+-oxidizers can use CH_4 in addition to NH_4^+ as a substrate:

CH_4 + O_2 + XH_2------>CH_3OH + H_2O + X

where X and XH_2 are co-enzyme and co-substrate in the reduced and oxidized form respectively. Some researchers (Alperin and Reeburg, 1985; Yavitt *et al.*, 1990; Kimura, 1992), have shown experimental evidence of the occurence of CH_4 oxidation in some anaerobic sediments. Since SO_4^{2-} is the only apparent oxidant present in sufficient quantity to cause a significant removal of CH_4 under these conditions, Yavitt *et al.* (1990) and Bartlett *et al.* (1987) suggested that SO_4^{2-}-reducers consume CH_4 under anaerobic conditions. Bartlett *et al.* (1987) observed that CH_4 and SO_4^{2-} concentrations are inversely related. This is consistent with the observation of Kimura *et al.* (1991), who reported that there is a competition of CH_4-producing and SO_4^{2-}-reducing bacteria for acetate and H_2-substrates. Papen and Rennenberg (1990) exemplified the possible reaction as follows:

CH_4 + SO_4^{2-}------------>HCO_3^- + HS^- $+H_2O$.

Methane emissions

Processes regulating CH_4 transport to the atmosphere

Net CH_4 emission is the difference between production and consumption. Three major processes have been identified to regulate the efflux of CH_4 from the soil/sediment into the atmosphere. These include, ebullition, molecular diffusion and vascular transport by plants. Ebullition occurs when the partial pressure of entrapped CH_4 within the soil exceeds the hydrostatic pressure; this results in the upward surge of the gas into the atmosphere when it reaches the soil/water interface. In the study of Bartlett *et al.* (1988), some 46-49% of the total CH_4 flux was attributed to ebullition, while Crill *et al.* (1988) reported values of up to 70%. The presence of vegetation moderates ebullition. For example, Takai and Wada (1990) observed that CH_4 ebullition is important during the early stage of flooding when rice plants were small, whereas vascular transport became more important as the rice plants grew. The diffusion of gases in water is about 10^4 times slower than in air, so that the exchange of gases almost stops when soils are waterlogged. Bartlett *et al.* (1985) reported that the CH_4 concentration gradient between sediments and water was a factor controlling rates of diffusive CH_4 flux in a Virginian swamp. Conrad and Rothfuss (1991), and Wahlen and Reeburgh (1990), observed that subsurface microbial CH_4 oxidation is important in controlling CH_4 emission by diffusion. Most wetland plant species develop the aerenchyma and intercellular gas space system in order to provide their roots with O_2. In doing so, the gas space system enables the transport of other gas species, including CH_4 and CO_2 from the soil/sediment to the atmosphere. This transport process could circumvent CH_4 oxidation by methanotrophs (Sebacher *et al.*, 1985; Schutz *et al.*,1991), but at the same time allow O_2 transport into the rhizosphere to provide oxic sites for CH_4 oxidation.

Methane emission from wetland environments

Rice paddies. Rice paddy fields are now considered to be the most important individual source of CH_4 (Schutz *et al.*, 1989), because of the anaerobic conditions created by flooding soils (Sass *et al.*, 1990). Currently, the total amount of CH_4 emitted from paddy fields on a global scale is about 60-146 Tg (Aselman and Crutzen, 1989). To meet the increasing global demand for rice, it is projected that production may increase to about 200 million ha by the year 2020 (IRRI, 1988). This would cause an increase in CH_4 emissions by over 20% (USEPA, 1990). Factors that influence CH_4 emissions from rice paddies are related to CH_4 oxidation, and processes controlling ebullition, molecular diffusion and transport through the plant (Schutz *et al.*, 1989). Among those factors include floodwater depth, vegetation, soil types and season of the year (Holzalpfel-Pschorn *et al.*, 1986; Schutz *et al.*, 1989). The mechanism of plant mediated CH_4 transport also influences emissions. For example, Nouchi *et al.* (1990) reported that CH_4 was mostly released from the macropores of rice culms, but not from the stomata of the leaf blades. They therefore found no correlation between transpiration and CH_4 emission rates.

Natural wetlands. Natural wetlands, including marshes, peatlands, tundras, fens, bogs and swamps, constitute the other natural ecosystem with strongly anaerobic conditions favorable for CH_4 production and emission. Similar to CH_4 emission measurements from paddy soils, the temporal and spartial variability in recorded CH_4 emissions from natural wetlands is large. A comprehensive review on CH_4 emissions from natural wetlands can be found in Bouwman (1990). The interpretation of the factors that may control CH_4 fluxes in these ecosystems is complicated by the extreme diversity in climatological, vegetational as well as soil conditions in these environments (Kyuma, 1985; Zoltai and Pollet, 1983; Pulliam and Meyer, 1992). Soil water content, temperature and other seasonal climatological factors as well as vegetation and land use are all potentially critical factors in determining whether natural wetlands function as a source or as a sink of atmospheric CH_4. Schutz *et al.* (1990a) obtained CH_4 emissions from natural wetlands of 100 ± 50 Tg yr^{-1} while Bolle *et al.* (1986) obtained values ranging from 25 to 70 Tg yr^{-1}. Other researchers (Bartlett *et al.*, 1987; Matthews and Fung, 1987; Harriss and Sebacher, 1981) reported a range of values for CH_4 emission from marshlands and some selected marshland plant species.

Importance of methane in global warming and climatic change

Methane, one of the major *greenhouse* gases, strongly influences the phytochemistry of the atmosphere (Cicerone and Oremland, 1988). The greenhouse gases (CO_2, CH_4, N_2O, Chlorofluorocarbons: (CFC), and water vapor) contribute to the so-called *greenhouse effect* or *global warming* in that they are fairly transparent to incoming shortwave solar radiation, but absorb and reflect longwave infrared radiation emitted from the warm surface of the Earth. Part of the energy associated with the longwave radiation that is reflected by the Earth's surface and lower atmosphere, is trapped within the atmosphere, thus keeping the Earth's surface temperature at a higher average value than it would have been if the greenhouse gas concentrations were low. Based on present calculations, CH_4 accounts for about 15 % of the current increase in commitment to global warming (Rodhae, 1990). Methane's strong ability to absorb infrared radiation and its relatively short atmospheric lifetime (8-12 years), combined with the fact that a large fraction of the atmospheric CH_4 originates from paddy soils, makes CH_4 control an important opportunity for addressing global climatic change. Taking into consideration the fact that the atmospheric CH_4 concentration is increasing at a much higher rate than atmospheric CO_2 concentration, and that an incremental molecule of CH_4 may trap about 32 times as much heat as as an incremental molecule of CO_2 (Dickson and Cicerone, 1986), methane is likely to become an even more important contributor to the greenhouse effect in the future, unless appropriate abatement measures are rapidly implemented. In addition to the direct greenhouse effect, CH_4 probably indirectly increases the rate of warming by positive feedback processes. Photochemical oxidation of CH_4 in the stratosphere increases the stratospheric water vapor concentration. Consequently, polar stratospheric cloud formation is increased and this enhances the destruction of the stratospheric ozone which is causing the so-called *antartic ozone hole* (Thompson, 1990; Mackenzie *et al.*, 1991). Other possible effects of global warming include sea-level rise leading to flooding in low-lying coastal areas and sea-water intrusion, redistribution of rainfall and drought in certain areas.

Plant functioning in response to soil reducing conditions.

Hormonal imbalances

Several researchers (Pereira and Kozlowski, 1977; Sena Gomes and Kozlowski, 1980a, b; Kawase 1981b; Kozlowski, 1982) reported that the magnitude of morphological response to flooding suggests growth-regulator mediation. Water stress induced by drought or waterlogging affects hormone balances which in turn control plant developmental patterns. The possibility of hormonal imbalance caused by root flooding was suggested by Phillip (1964). Root death prevents synthesis of cytokinin and its subsequent translocation to the shoots and leaves. It was demonstrated (Phillip, 1964) that there is a presence of substantially greater amounts of auxin in the shoots of flooded plants compared to nonflooded plants. Evidence, indicating changes in gibberellin (Reid and Crozier, 1971), ethylene (Kawase, 1978; Newsome *et al.*, 1982; Tang and Kozlowski 1982; Jackson, 1988; Abeles, 1992), and absissic acid (ABA) (Hiron and Wright, 1973), and reduced synthesis of gibberrllin and indole-acetic acid (IAA) in roots (Reid and Crozier, 1971) have been reported in the literature. Elevated ethylene concentration has been found under hypoxic conditions (Kawase,1978; Jackson, 1988). The effects of an increase in ethylene concentration on roots include enhancement of aerenchyma formation in some species (Drew *et al.*, 1979; Kawase 1981a; Justin and Armstrong, 1991). Ethylene also inhibits root elongation in some species (Robbins *et al.*, 1985; Riov and Yang, 1989), and promotes stem elongation in others (Metraux and Kende, 1983). Such promotion has been reported to result from enhanced cell growth, increase in cell numbers, and increased cell wall acidification (Metraux and Kende, 1983).

Physiological changes

Early stomatal closure and reduction in photosynthetic rate are among the known documented effects of soil anaerobiosis on plants (Newsome *et al.*, 1982; Pezeshki and Chambers, 1985 a; b). For example, rapid closure of stomata in *Fraxinus pennsylvanica* seedlings was attributed to a high CO_2 concentration in substomatal cavities of flooded plants and/or production of ABA by leaf cells (Sena Gomes and Kozlowski, 1980a). There is some evidence that stomata adapt to flooding by opening after a certain period of flooding (Regehr *et al.*, 1975; Kozlowski and Pallardy, 1979; Sena Gomes and Kozlowski, 1988a). Reductions in photosynthesis in response to flooding have been reported in several wetland species. For example, Pezeshki *et al.* (1989) reported that *Spartina alterniflora* showed decreased net photosynthesis in response to intensity of soil redox potentials, followed by recovery, thus indicating that *S. alterniflora* could adjust somewhat to the reduced conditions. DeLaune *et al.* (1984) showed that a decline in soil Eh from +550 to -210 mV resulted in a 21% reduction in net photosynthesis.

Root growth

According to some researchers (Jackson and Drew, 1984; John and Greenway, 1976; Atwell and Greenway 1987), normal growth and functioning of roots require more O_2 than do the root respiration processes. Under flooded conditions, O_2 must reach the roots through air spaces from aerial parts (Armstrong, 1972; 1979). An initial increase in ADH (Alcohol dehydrogenase) activity followed by decrease in ADH activity in some flood-tolerant plants during extended flooding has been attributed to aerenchyma development (Keeley, 1979; Burdick, 1988; 1989). Pezeshki and DeLaune (1990) reported that *Spartina patens* grown in an anaerobic rhizophere had approximately three times as much root ADH activity as roots of plants maintained at high soil Eh.

Oxygen supply is essential for root elongation in both flood-tolerant and flood-sensitive plants (Atwell *et al*, 1985; Topa and McLeod, 1986; Pezeshki and DeLaune, 1990). The status of soil aeration influences root O_2 supply because of changes in gradient of O_2 concentration between aerial parts and rhizosphere (Yamasaki, 1987). Two types of root growth modification under anaerobic conditions have been reported in the literature: increased branching of the roots (Kleinendorst and Brower, 1967) and adventitious root formation (Kramer, 1951; Alberda, 1953). Adventitious roots are very important, because they enhance O_2 transport, can tolerate high levels of CO_2 (Hook *et al.*, 1971) and efficiently absorb water (Kozlowski, 1982; 1984).

Aerenchyma formation

Mechanism

The oxygen requirements of roots and rhizomes of wetland plants are largely met by transport of O_2 from the atmosphere through the gas phase continuum of the plant tissue (Conway, 1940; Van Raalt, 1940; Crawford, 1992). The development of aerenchyma and related internal pathways for O_2 diffusion to roots is a major adaptation mechanism in wetland plants (Hook and Scholtens, 1978, Pezeshki *et al.*, 1991). Smirnoff and Crawford (1983) define aerenchyma as any tissue that contains large, air-filled intercellular space or lacunae. Roots develop the gas-filled spaces (aerenchyma) which interconnect longitudinally and join with the gas spaces of the stem base (Erdmann *et al.*, 1988), thus providing a pathway of low resistance for the diffusion of O_2 from the air. A good estimation of root aerenchyma formation is the measurement of the porosity (Jensen *et al.*, 1969).

Reported physiological changes in plants prior to aerenchyma development following flooding may include an increase in ADH activity (John and Greenway, 1976), and increase in the production of ethylene precursor, 1-amino-cycloproprane carbonylic acid (ACC) and its conversion into ethylene (Drew *et al.*, 1979; Jackson *et al.*, 1988); an increase in the level of ABA and IAA, leading to stomatal closure, a decrease in the quantity of cell-wall producing enzymes and an increase in cellulase levels (De Wit, 1983; Marschner, 1986). Two forms of aerenchyma formation have been identified: *lysigenous aerenchyma* and *schizogenous aerenchyma* (Kawase, 1981a; Smirnoff and Crawford, 1983; Justin and Armstrong, 1987). Lysigenous aerenchyma is formed by various degrees of cell wall separation and cell disintegration (lysis) of cortical cells. The sequential steps in lysigenous aerenchyma development as described by Kawase (1981a) may be represented as follows: flooding results in hypoxia/anoxia leading to the synthesis of ACC (ethylene precursor) and its conversion to ethylene, which in turn increases cellulase activity, and subsequently cell lysis and aerenchyma formation. Schizogenous aerenchyma formation, on the other hand, involves the enlargement of intercellular spaces without cell collapse (Laan *et al.*, 1989).

Although in general aerenchyma formation is induced or enhanced by O_2-limiting conditions, instances of aerenchyma formation in some plant species such as *Eriophorum* (Smirnoff and Crawford, 1983) and *Rumex* (Laan *et al.*, 1989), under well aerated conditions have been reported.

Importance of aerenchyma in root metabolism and rhizosphere oxygenation

Perhaps the most significant long-term adaptation of wetland species to soil anaerobiosis is the development of the aerenchyma tissue in the root and shoot to assume the role of the external gas diffusion path once provided by the soil atmosphere (Hook and Scholtens, 1978; Armstrong, 1979). Root oxygenation in flooded plants is essential in the maintenance of aerobic respiration in the absence of which the less efficient energy yielding anaerobic fermentation results. In experiments with *Zea mays* and *Oryza sativa,* decreasing supplies of O_2 below the critical oxygen pressure (COP: the oxygen pressure below which aerobic respiration is reduced) resulted in depression of the root energy status (Saglio *et al.*, 1984) as indicated by the adenylate energy charge ratio (AEC: the ratio of phosphorylated adenine nucleotides to the adenine nucleotide pool). The other function of the aerenchyma-facilitated O_2 pathway to roots that has been related to wetland survival is the ability to provide an oxidizing atmosphere at the root surface for oxidation. Rhizosphere oxygenation by O_2 leakage from roots is of great importance since by this mechanism, potential soil toxins, particularly reduced forms of Fe, Mn and H_2S, which could increase to toxic levels under anaerobic conditions (Armstrong, 1971, 1992; Ottow *et al.*, 1982), are immobilized or neutralized. At only 2.5 ppm, H_2S kills roots (Culbert and Ford, 1972). Oxygen diffusion from aerial parts to the roots and the subsequent sulfide oxidation in the rhizosphere have been considered major mechanism allowing a high level of sulfide tolerance in some species (Teal and Kanwisher, 1966). Evidence of O_2 diffusion outward from roots is derived from observations of the precipitation of oxidized Fe^{3+} and changes in redox conditions close to root surfaces (Chen *et al.*, 1980; Taylor *et al.*, 1984), and by direct measurements of O_2 released by roots in vitro.

REFERENCES TO INTRODUCTION AND LITERATURE REVIEW

Abeles, F.B. 1992. Ethylene in Plant Biology. Academic Press, Inc. New York.

Alberda, Th. 1953. Growth and root development of lowland rice and its relation to oxygen Supply. Plant Soil 5:1-28.

Alperin, M.J., and W.S. Reeburg. 1985. Inhibition experiments on anaerobic methane oxidation. Appl. Environ Microbiol. 50:940-945.

Armstrong, W. 1979. Aeration in higher plants. In: Advances in Botanical Research (Ed. H.W. Woolhouse) pp. 697. Academic Press. London.

Armstrong, W. 1972. A re-examination of the functional significance of aerenchyma. Physiol. Plant. 27:172-177.

Armstrong, W. 1971. Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration and waterlogging. Physiol. Plant. 25:192-198.

Armstrong, J., W. Armstrong, and P. M. Beckett. 1992. *Phragmites australis:* Venturi- and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. New Phytol. 120:197-207.

Aselman, I., and P.J. Crutzen. 1989. Freshwater wetland: Global distribution of natural wetlands and rice paddies, their net primary productivity, seasonality and possible methane emissions. J. Atmos. Chem. 8:307-358.

Atwell, B.J., and H. Greenway. 1987. The relationship between growth and oxygen uptake in hypoxic rice seedlings. J. Exp. Bot. 38:454-465.

Atwell, B. J., C.J. Thompson, H. Greenway, G. Wards and I. Waters. 1985. A study of the impaired growth of roots of *Zea mays* seedlings at low oxygen concentrations. Plant, Cell & Envir. 8:179-188.

Barker, H.A. 1956. Bacterial fermentations. Wiley, New York, p. 1-95.

Bass Becking, L.G.M., I.R. Kaplan, and D. Moore. 1960. Limits of the natural environment in terms of pH and oxidation-reduction potentials. J. Geology. 68:243-284.

Bartlett, K.B., P.M. Crill, D.I. Sebacher, R.C. Harris, J.O. Wilson, and J.M. Melack. 1988. Methane fluxes from the central Amazonian floodplain. J. Geophy. Res. 93:1571-1582.

Bartlett, K.B., R. C. Harris, and D.I. Sebacher. 1985. Mathane flux from coastal salt marshes. J. Geophy. Res. 90:5710-5720.

Bartlett, K.B., D.S. Bartlett, R.C. Harris, and D.I. Sebacher. 1987. Methane emissions alon a salt marsh salinity gradient. Biogeochem. 4:183-202.

Bohn, H.L. 1968. Electromotive force of inert electrodes in soil suspensions. Soil Sci. Soc. Am. Proc. 32:211-215.

Bolle, H.J., W. Seiler, and B. Bolin. 1986. Other greenhouse gases and aerosols: assessing their role for atmospheric radiative transfer. In: The greenhouse effect climatic change and ecosystems (eds B.Bolin, B.R. Doos, B.A. Warrick and D. Jager). SCOPE 29, John Wiley and Sons, New York. p. 157-203.

Boon, P.I., and B.K. Sorrell. 1991. Biogeochemistry of billabong sediments. I. The effect of macrophytes. Freshwater Biology 26:209-226.

Bouwman, A.F. 1991. Agronomic aspects of wetland rice cultivation and associated methane emissions. Biogeochem. 15:65-88.

Bouwman, A.F. (ed.), 1990. Soils and the greenhouse effect. John Wiley and Sons, Chichester.

Burdick, D.M. 1989. Root aerenchyma development in *Spartina patens* in response to flooding. Am. J. Bot. 76:777-780.

Burdick, D.M. 1988. The relationship between root anatomic and metabolic responses to soil waterlogging in coastal grass *Spartina patens*. Ph.D. Dissertation, Louisiana State Univ., Baton Rouge.

Burdick, D.M., and I.A. Mendelssohn. 1990. Relationship between anatomical and metabolic responses to soil waterlogging in the coastal grass *Spartina patens*. J. Exp. Bot. 41:223-228.

Cappenberg, T.E. 1975. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. I. Field observations. Antonie van Leeuwenhoek. J. Microbiol. 40:285-295.

Chen, C.C., J.B. Dixon, and F.T. Turner. 1980. Iron coatings on rice roots: morphology and models of development. Soil Sci. Soc. Am. J. 44:1113-1119.

Cicerone, J.D. and Oremland. 1988. Biogeochemical aspects of atmospheric methane. Global Biogeochem. Cycles 2:299-327.

Cicerone, J.D. Shetter, and C.C. Delwiche. 1983. Seasonal variation of methane flux from a Californian rice paddy. J. Geophys. Res. 88:11,022-11,024

Conrad, R. and F. Rothfus. 1991. Methane oxidation in the soil surface layer of flooded rice field and the effect of ammonium. Biol. Fert. Soils 12:28-32.

Conway, V.M. 1940. Studies on the autecology of *Cladium mariscus* R. Br. Part III. The aeration of the subteranean parts of the plant. New Phytol. 35:359-364.

Crawford, R.M.M. 1992. Availability as an ecological limit to plant distribution. Ad. Ecol. Res. 23:93-185.

Crill, P.M. , K.B. Bartlette, R.C. Harris, E. Gorham, E.S. Verry, D.I. Sebacher, L. Madzar, and W. Sanner. 1988. Methane flux from Minnesota peatlands. Global Biogeochem. Cycles 2:371-384.

Culbert, D.L., and H.W. Ford. 1972. The use of multi-celled apparatus for anaerobic studies of flooded root systems. Hort. Sci. 71:29-31.

DeLaune, R.D., W.H. Patrick Jr., and J.M. Brannon. 1976. Nutrient transformations in Louisiana salt marsh soils. coastal zone. Mngt. Series Publication No. LSU-T-76-009.

DeLaune, R.D., and S.R. Pezeshki. 1991. Role of soil chemistry in vegetative ecology of wetlands. Trends in Soil Science. 1:101-113.

DeLaune, R.D., S.R. Pezeshki, and J.H. Pardue. 1990. An oxidation-reduction buffer for evaluating the physiological response of plants to root oxygen stress. Environ. Exp. Bot. 30:243-247.

DeLaune, R.D., C.J. Smith, and M.D. Tolley. 1984. The effect of sediment redox potential on nitrogen uptake, anaerobic root respiratio and growth of *Spartina alterniflora*. Loisel. Aquatic Bot. 18:223-230.

De Wit, M.C.J. 1983. Morphology and function of roots and shoot growth of crop plants under oxygen deficiency. In: Plant Life in Anaerobic Environments. (Ed.) D.D. Hook and R.M.M. Crawford. Ann Arbor, Mich.

Dickson, R.E., and R.J. Cicerone. 1986. Future global warming from atmospheric trace gases. Nature 319:109-114.

Drew, M.C., M.B. Jackson, and S. Giffard. 1979. Ethylene-prompted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in Zea mays. Planta 147:83-88.

Drew, M.C., P.H. Saglio, and A Pradet. 1985. Larger adenylate energy charge and ATP/ADP rotios in aerenchymatous roots of Zea mays in anaerobic media as a consequence of improved internal oxygen transport. Planta 165:51-58.

Dunfield, P., R. Knowles, R. Dumont, and T.R. Moore. 1993. Methane production and consumption in temperate and subartic peat soils: Response to temperature and pH. Soil Biol. Biochem. 25:3210326.

Erdmann, B. E.M. Wieldenroth, and D. Ostareck. 1988. Anatomy of the root-shoot junction in wheat seedlings with respect to internal oxygen transport and root growth retardation by external oxygen shortage. Ann. Bot. 62:521-529.

Gambrell, R.P., and W.H. Patrick. 1978. Chemical and microbiological properties of anaerobic soils and sediments. In: Plant Life in Anaerobic Environments. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan. 375-423.

Garcia, J.L. 1990. Taxonomy and ecology of methanogens. FEMS Microbiol. Rev. 87:297-308.

Glinski, J., and W. Stepniewslki. 1983. Soil aeration and its role for plants. CRC Press, Inc., Boca Raton, Florida. pp.173-188.

Harris, R.C., and D.I. Sebacher. 1981. Methane flux in forested freshwater swamps of the southeastern United States. Geophy. Res. Letters 9:1002-1004.

Hiron, R.W.P., and S.T.C. Wright. 1973. The role of endogenous abscisic acid in the response of plant stress. J. Exp. Bot. 24:769-781.

Holzapfel-Pschorn, A., R. Conrad, and W. Seiler. 1986. Effects of vegetation on the emission of methane from submerged paddy soil. Plant Soil 92:223-233.

Hook, D.D., C.L. Brown, and P.P. Kormanik. 1971. Inductive flood tolerance in swamp tupelo. J. Exp. Bot. 22:78-89.

Hook, D.D., and J.R. Scholtens. 1978. Adaptations and flood-tolerance of tree species. In: Plant Life in Anaerobic Environments. (eds. D.D. Hook and R.M.M. Crawford) p.299-332. Ann Arbor Science, Ann Arbor, Michigan.

IRRI (International Rice Research Institute). 1988. World Rice Statistics, 1987. Los Banos, Philippines.

Jackson, M.B. 1988. Involvement of the hormones ethylene and abscisic acid in some adaptive responses of plants to submergence, soil waterlogging and oxygen storage. In: The Ecology and Management of Wetlands. pp.373-382.

Jackson, M.B., and M.C. Drew. 1984. Effects of flooding on growth and metabolism of herbaceous plants. In: Flooding and plant growth (ed. T.T. Kozlowski) pp. 47-128. Academic Press, Inc.

Jenson, C.R., R.J. Luxmoor, S.D. Van Gundy, and L.H. Stolzy. 1969. Root air-space measurements by a pycnometer method. Agron. J. 61:474-475.

John, C.D., and W. Greenway. 1976. Alcoholic fermentation and activity of some enzymes in rice roots under anaerobiosis. Aust. J. Pl. Physiol. 3:325-336.

Justin, S.H.F., and W. Armstrong. 1991. Evidence for the involvement of ethylene in aerenchyma formation in adventitious roots of rice. New Phytol. 118:49-62

Justin, S.H.F., and W. Armstrong. 1987. The anatomical characteristics of roots and plant response to soil flooding. New Phytol. 106:465-495.

Kawase, M.J. 1981a. Anatomical and morphological adaptations of plants to waterlogging. Hort. Sci. 16:30-34.

Kawase, M.J. 1981b. Effect of ethylene on aerenchyma development. Am. J. Bot. 68:652-658.

Kawase, M.J. 1978. Anaerobic elevation of ethylene concentration in waterlogged plants. Am. J. Bot. 65:736-740.

Keeley, J.E. 1979. Population differentiation along a flood frequency gradient: Physiological adaptation to flooding in *Nysa sylvastica*. Ecol. Monogr. 49:89-108.

Kimura, M. 1992. Methane emissions from paddy soils in Japan and Thailand. In: World Inventory of Soil Emission Potentials (eds N.H.Batjes and E.M. Bridges). Proceedings of an international workshop organized in the framework of the Netherlands National Research Programme on Global Air Pollution and Climate Change (24-27 August 1992). WISE Report No. 2, International Soil Reference and Information Centre, Wageningen.

Kimura, M., H. Murakami, and H. Wada. 1991. CO_2, H_2 and CH_4 production in rice rhizosphere. Soil Sci. Pl. Nutri. 37:55-60.

Kimura, M., H. Wada, and Y. Takai. 1984. Studies on the rhizosphere of paddy rice. IX. Microbial activities in the rhizosphere of paddy rice. J. Sci. Manure, Jpn. 55:338-343 (in Japanese).

Kleinendorst, A., and R. Bromer. 1967. Responses of two different clones of perennial ryegrass to aeration of the nutrient solution. Jaarb. I.B.S. 29-39

Kozlowski, T.T. 1984. Plant responses to flooding of soil. Biosci. 34:162-167.

Kozlowski, T.T. 1982. Water supply and tree growth. Part II. Flooding. For. Abst. 43:145-161.

Kozlowski, T.T., and S.G. Pallardy. 1979. Stomatal responses of *Fraxinus pennsylvanica* seedlings during and after flooding. Physiol. Plant. 46:155-158.

Kramer, P.J. 1951. Causes of Injury to Plants from Flooding of Soil. Plant Physiol. 16:722-736.

Kyuma, K. 1985. Fundamental characteristics of wetland soils. In: Wetland soils: Characterization, classification and utilization. International Rice Research Institute, Los Banos.

Laan, P., A. Smolders, C.W.P.M. Blom, and W.Armstrong. 1989. The relative roles of internal aeration, radial oxygen losses, iron exclusion and nutrient balances in flood-tolerance of *Rumex* species. Acta Bot. Neerl. 38(2) p. 131-145.

Lindau, C.W. , P.K. Bollick, R.D. DeLaune, W.H. Patrick, Jr., and V.J. Law. 1991. Effect of urea fertilizer and environmental factors on CH_4 emissions from a Louisiana USA, rice field. Plant Soil 136:195-203.

Lindau, C.W., W.H. Patrick, Jr., and R.D. DeLaune. 1993. Factors Affecting Methane Production in Flooded Rice Soils. In: Gricultural Ecosystem.Effects on Trace Gases and Global Climatic Change. ASA Special Publication no. 55. Madison, WI.

Lovely, D.R., and M.J. Klug. 1983. Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. Appl. Environ. Microbiol. 45:187-192.

MacKenzie, A.R., R.M. Harrison, I. Colbeck, and C.N. Hewitt. 1991. The role of biogenic hydrocarbons in the production of ozone in urban plumes in southeast England. Atmospheric Environ. 25:351-359.

Marschner, H. 1986. Mineral Nutrition in Higher Plants. Academic Press, London.

Matthews, E., and I. Fung. 1987. Methane emissions from natural wetlands: Global distribution, area, and environmental characteristics. Global Biogeochem. Cycles. 1:61-86.

Metraux, J.P., and H. Kende. 1983. The role of ethylene in the growth response of submerged deepwater rice. Plant Physiol. 72:441-446.

Newsome, R.D., T.T. Kozlowski, and Z.C. Tang. 1982. Responses of *Ulmus americana* seedlings to flooding of soil . Can. J. Bot. 60:1688-1695.

Nouchi, I. S. Mariko, and K. Aoki. 1990. Mechanism of methanetransport from the rhizosphere to the Atmosphere through rice plants. Plant Physiol. 94:59-66.

Oremland, R.S. 1988. Biogeochemistry of methanogenic bacteria. p. 641-705. In: A.J.B. Zehnder (ed.) Biology of anaerobic microorganisms. John Wiley and Sons., New York.

Ottow, J.C.G., G. Benciser, and I Watanabe. 1982. Iron toxicity of rice as a multiple nutritional soil stress. Trop. Agr. Res. Ser. 15:167-179.

Papen, H., and Rennenberg. 1990. Microbial processes involved in emissions of radiatively important trace gases. In: Transactions 14th International Congress of Soil Science, Kyoto II:232-237.

Parasher, D.C., J. Rai, K. Gupter, and N. Singh. 1991. Parameters affecting methane emission from paddy fields. Ind. J. Radio Space Phys. 20:12-17

Patrick, W.H., Jr., and I.C. Mahaptra. 1968. Transformation and availability to rice plant of nitrogen and phosphorus in waterlogged soils. Ad. Agron. 20:323-359.

Patrick, W.H., Jr., and C.N. Reddy. 1978. Chemical changes in rice soils. p. 361-379. In: Soils and Rice. International Rice Research Institute, Los Banos, Philippines.

Pereira, J.S., and T.T. Kozlowski. 1977. Variation among woody angiosperms in response to flooding. Physiol. Plant. 41:184-192.

Pezeshki, S.R., and J.L. Chambers. 1985a. Stomatal and photosynthetic response of sweetgum (*Liquidamber styraciflua L.*) to flooding. Can J. For. Res. 15:371-375.

Pezeshki, S.R., and J.L. Chambers. 1985b. Response of cherrybark oak (*Quercus falcata* var. *pagodaefolia*) seedlings to short-term flooding. For. Sci. 31:760-771.

Pezeshki, S.R., and R.D. DeLaune. 1990. Influence of sediment oxidation-reduction on root elongation in *Spartina patens*. Acta Oecologia 11:377-383.

Pezeshki, S.R., R.D. DeLaune, and W.H. Patrick, Jr. 1989. Effect of fluctuating rhizosphere redox potential on carbon assimilation of *Spartina alterniflora.* Oecologia 80:132-135.

Pezeshki, S.R., S.W. Matthews, and R.D. DeLaune. 1991. Root cortex structure and metabolic response of *Spartina patens* to soil redox conditions. Envir. Exp. Bot. 31(1):91-97.

Phillip, I.D.J. 1964. Root-Shoot Hormone Relations. I. The importance of an aerated root system in the regulation of growth hormone levels in the shoot of *Helianthus annus*. Ann Bot. N.S. 28:17-35.

Ponnamperuma, F.N. 1984. Effects of flooding on soils. In:Flooding and plant growth (ed. T.T. Kozlowski). Academic Press Inc. p. 9-43.

Ponnamperuma, F.N..1972. The chemistry of submerged soils. Adv. Agron. 24:29-89.

Pulliam, W.M., and J.L. Meyer. 1992. Methane emissions from floodplain swamps of the Ogeechee River: long-term patterns and effects of climate change. Biogeochem. 15:151-174.

Reddy, K.R., T.C. Feitjel, and W.H. Patrick, Jr. 1986. Effect of soil redox conditions on microbial oxidation of organic matter. In: The Role of Organic Matter in Modern Agriculture. (Eds.) Y. Chen and Y Avnimelech. Martinus Nijhoff Publ. pp. 117-156.

Regehr, D.L., F.A. Bazzaz, and W.R. Boggess. 1975. Photosynthesis, transpiration and leaf conductance of *Populus deltoides* in relation to flooding and drought. Photosynthetica 9:52-61

Reid, D.M., and A. Crozier. 1971. Effects of waterlogging on the gibberellin content and growth of tomato plants. J. Exp. Bot. 22:39-48.

Riov, J., and S.F. Yang. 1989. Ethylene and auxin-ethylene interaction in adventitious root formation in mung bean (*Vigna radiata*) cuttings. J. Pl. Growth Regul. 8:131-141.

Robbins, J.A., M.S. Reid, J.L. Paul, and T.L Rost. 1985. The effect of ethylene on adventitious root formation in mung bean (*Vigna radiata*) cuttings. J.Pl. Growth Regul. 4:147-157.

Rodhae, H. 1990. A comparison of the contribution of various gases to the greenhouse effect. Science 248:1217-1219.

Saglio, P.H., M.C. Drew, and A. Pradet. 1988. Metabolic acclimation to anoxia induced by low (2-4 kPa partial pressure) oxygen pretreatment (hypoxia) in roots of *Zea mays*. Plant Physiol. 86:61-66.

Saglio, P. H., M.Rancillac, R.Bruzan, and A. Pradet. 1984. Critical oxygen pressure for growth and respiration of excised and intact roots. Plant Physiol. 76:151-154.

Sass, R.L., F.M. Fisher, P.A. Harcombe, and F.T. Turner. 1990. Methane production and emission in agricultural wetlands. Global Biogeoche. Cycles. 4:47-68.

Schat, H. 1984. A comparative ecophysiological study on the effects of waterlogging and submergence on dune slack plants: growth, survival and mineral nutrition in sand culture experiments. Oecologia 62:279-286.

Schutz, H., P. Schroder, and Rennenberg. 1991. Role of plants in regulating methane flux to the atmosphere. p. 29-63. In T. Sharkey et al. (ed.) Trace gas emissions by plants. Academic Press, New York.

Schutz, H. P., W. Seiler and R.Conrad. 1990a. Soil and Land use related sources and inks of methane in the context of the global methane budget. In: Soil and the greenhouse effect (ed. A.F. Bouwman). John Wiley and Sons, Chichester. p. 269-285.

Schutz, H. P., W. Seiler, and R. Conrad. 1990b. Influence of soil temperature on methane emission from paddy fields. Biogeochem. 11:77-95.

Schutz, H. P., W. Seiler, and R. Conrad. 1989. Processes involved in formation and emission of methane in rice paddies. Biogeochem. 7:33-53.

Sebacher, D.I., R.C. Harriss, and K.B. Bartlett. 1985. Methane emissions to the atmosphere through aquatic plants. J. of Environ Qual. 14:40-46.

Sena Gomes, A.R., and T.T. Kozlowski. 1980a. Growth response and adaptation of *Fraxinus pennsylvanica* seedlings to flooding. Plant Physiol. 66:267-271.

Sena Gomes, A.R., and T.T. Kozlowski. 1980b. Responses of *Melaleuca quinquenervia* seedlings to flooding. Physiol. Plant. 49:373-377.

Seiler, W., A. Holzapfel-Pschorn, R. Conrad, and D. Scharffe. 1984. Methane emissions from rice paddies. J. Atm. Chem. 1:241-268.

Smirnoff, N., and R.M.M. Crawford. 1983. Variation in the structure response to flooding of root aerenchyma in some wetland plants. Ann. Bot. 51:237-249.

Smith, K.A. and M.B. Jackson. 1974. Ethylene, waterlogging and plant growth. Agric. Res. Council Letcombe Lab. Ann. Rep. 1973. pp. 60-75.

Steudler, P.A., R.D. Bowden, J.M. Mellilo, and J.D. Aber. 1989. Influence of nitrogen fertilization on methane uptake in temperate forest soils. Nature 341:314-316.

Stumn, W., and J.J. Morgan. 1981. Aquatic chemistry: An Introduction Emphasizing Chemical Equilibria in Natural Waters, 2nd ed. John Wiley and Sons, New York.

Takai, Y., and E. Wada. 1990. Methane formation in waterlogged paddy soils and its controlling factors. In: Soils on a warmer Earth (ed. H.W. Scharpensel).

Tang, Z.C., and T.T. Kozlowski. 1982. Physiological, morphological, and growth responses of *Platanus occidentalis* seedlings to flooding. Plant Soil 66:243-255.

Taylor, G.J., A.A. Crowder and R. Rodden. 1984. Formation and morphology of an iron plaque on the roots of *Typha datifolia* grown in solution culture. Am. J. Bot. 71:666-675.

Teal, J.M., and J.W. Kanwisher 1966. Gas transport in the marsh grass *Spartina alterniflora*. J. Exp. Bot. 17:355-361.

Thompson, A.M. 1990. Effects of atmospheric chemical and climate change on tropospheric ozone. Ozone Sci. Eng. 12:177-194.

Topa, M.A., and K.W. Mccleod. 1986. Aerenchyma and lenticel formation in pine seedlings: A possible avoidance mechanism to anaerobic growth conditions. Physiol. Plant. 68:540-550.

Turner, F.T., and W.H. Patrick, Jr. 1968. "Chemical changes in waterlogged soils as a result of oxygen depletion, " Trans. 9th Int. Congr. Soil Sci., Adeilade, Australia. 4:53-65.

USEPA. (United States Environmental Protection Agency). 1991. Introduction: Methane, global warming, and wetland rice. p. 12-18. In B.V. Braatz and K.B. Hogan (ed.) Sustainable rice productivity and methane reduction research plan. USEPA. Office of Air and Radiation, Washington, DC.

USEPA. (United States Environmental Protection Agency). 1990. Methane emissions and opportunities for control. EPA/400/9-90/007. USEPA, Washington, DC.

Van Breemen, N., and T.C.J. Feijtel. 1990. Soil processes and properties involved in the production of greenhouse gases, with special relevance to soil taxonomic systems. In Soils and the greenhouse effect (ed. A.F. Bouwman). John Wiley and Sons, Chichester. p. 195-223.

Van Raalt, M.H. 1940. On the oxygen supply of rice roots. Ann. Bot. Gardens Buitenzorg 51:43-57.

Wahlen, S.C., and W.S. Reeburg. 1990. Consumption of atmospheric methane by tundra soils. Nature 346:160-162.

Wang, Z., R.D. DeLaune, P.H. Masscheleyn, and W.H. Patrick, Jr. 1993. Soil redox and pH effects on methane production in a flooded rice soil. Soil Sci. Soc. Am. J. 57:382-385.

Wetermann, P., and B.K. Ahring. 1987. Dynamics of methane production, sulfate reduction, and denitrification in a permenently waterlogged alder swamp. Appl. Environ. Microbiol. 53:2554-2559.

Wolin, M.J., and T.L. Miller. 1987. Interspecies hydrogen transfer: 15 years later. ASM News 48:561-565.

Yagi, K., and K. Minami. 1990. Effects of organic matter application on CH4 emissions from Japanese paddy fields. pp. 467-573. In: Soils and the greenhouse effect (ed. A.F. Bouwman). John Wiley and Sons, New York.

Yamasaki, S. 1987. Oxygen demand and supply in *Zizania latifolia* and *Phragmites australis*. Aquat. Bot. 29:205-215.

Yavitt, J.B., D.M. Downey, E. Lancaster, and G.E. Lang. 1990. Methane consumption in decomposing *Sphagnum* derived peat. Soil Biol. Fert. 22:441-447.

Zoltai, S.C., and F.C. Pollet. 1983. Wetlands in Canada: Their classification, distribution and use. pp.245-268. In: Ecosystems of the world (4B). Mires: Swamps, Bog, Fen and Moor (ed. A.J.P. Gore), Elsevier, Armsterdam.

CHAPTER 1

A COLORIMETRIC METHOD FOR ASSAYING DISSOLVED OXYGEN LOSS FROM CONTAINER-GROWN RICE ROOTS

INTRODUCTION

Methods of soil solution O_2 assay include the use of platinum wire electrodes (Boon and Sorrell, 1991), paramagnetic oxygen analysis, volumetric, polarographic, gas chromatography, mass-spectrometry, and colorimetric techniques (Glinski and Stepniewski, 1983). Some of these techniques have been used to study the oxidizing power of rice roots. Oxygen release by roots in a deoxygenated liquid medium was shown by colorimetric and polarographic analyses (Armstrong, 1967; Luxmoore *et al.*, 1972). Most of the colorimetric techniques are modifications of the manganous hydroxide-potassium iodide method of Winkler (1888) or the indigo-carmine dye method (Armstrong, 1967). These colorimetric methods have been criticized for their lack of accuracy and/or the slow rates of reaction with reagents in the presence of O_2. For instance, loss of iodine vapors are reported to cause appreciable errors in the Winkler method while in the indigo carmine dye method (Armstrong, 1967), elemental sulfur from H_2S causes interference with spectrophotometric readings; in addition, the sulfides could be toxic to living tissue.

Titanium (III) is a strong reducing agent and Ti^{3+}-citrate is purple blue in solution. Autoxidation of Ti^{3+} eliminates O_2 from a medium by a first order reaction with a rate constant, $k=11.4 + (0.8 \times 10^{-5}) s^{-1}$ at 25°C (DeLaune *et al.*, 1990; Johnson *et al.*, 1957).

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The Ti^{3+}-citrate solution provides a strictly de-oxygenated oxygen-scavenging medium around plant roots and becomes colorless when oxidized to Ti^{4+}-citrate. Zehnder and Wuhrman (1976) successfully used Ti^{3+}-citrate at varied concentrations as a non-toxic oxidation-reduction buffering system for culturing obligate anaerobes; they reported that the Ti^{3+}-citrate was not metabolized by the organisms and remained intact during growth.

Roots and rhizomes of wetland plants receive their oxygen supply largely from the atmosphere through the gas phase continuum of the plant tissue (Van Raalt, 1940). This O_2 transport serves the dual purpose of maintaining aerobic metabolism of roots and microbes, and oxidizing substances that are toxic in the reduced state (Armstrong, 1971; 1979). Potential phytotoxins such as Fe^{2+}, Mn^{2+}, H_2S and certain organic acids may be oxidized to harmless or insoluble compounds upon contact with the oxidized rhizosphere. Evidence of O_2 diffusion outwards from roots is derived from observations of the precipitation of oxidized Fe^{3+} and changes in redox conditions close to root surfaces (Taylor *et al.*, 1984).

Flooding induces a number of responses in plant roots of which aerenchyma formation is one of the most obviously adaptive (Justin and Armstrong, 1987). Air diffuses from leaves through aerenchyma tissue to the submerged parts of plants (Sale and Wetzel, 1983). Aerenchyma is any tissue containing large air-filled intercellular spaces or lacunae. Growth of cultivated and marshland species under flooded conditions or in solution culture with low O_2 concentration usually increases the amount of air space in roots (Armstrong, 1971; Smirnoff and Crawford, 1983; Pezeshki *et al.*, 1991). On the contrary, John *et al.* (1974) and Jackson *et al.* (1985) observed that rapid vertical degeneration of the root cortex of rice occurs even under aerated conditions. They suggested that aerenchyma formation is part of ordinary root growth and not increased by poorly aerated surroundings, although cultivars may respond differently.

Although not easily measured, O_2 release from rice roots determines the plant's ability to survive reducing conditions. DeLaune *et al.* (1990) used the oxidized (Ti^{4+}) and reduced (Ti^{3+}) forms of titanium-citrate to study the photosynthetic activity of *Zea mays* and *Spartina patens*, and postulated that Ti^{3+}-citrate solution could be used to quantify O_2 transport through aerenchyma to the rhizosphere. The objective of the present study was to test the Ti^{3+}-citrate complex for its potential to quantify dissolved O_2 concentrations in solutions bathing the whole root system of container-grown rice roots. The proposed technique was compared with a polarographic method. The study also sought to elucidate the extent to which soil aeration status could influence root porosity and the concomitant oxygen release into the rhizosphere of seven US rice cultivars.

MATERIALS AND METHODS

Plant Material

Seeds of seven rice cultivars: *Katy, Lemont, Tebonnet, Millie, Mars, Rico and Mercury* were soaked in 0.1% $HgCl_2$ for 60 s, rinsed with tap water and allowed to germinate in petri-dishes. They were then transferred to aluminum trays containing air-dried sand and half-strength nutrient solution containing 20 mg L^{-1} N as NH_4NO_3, 5 mg L^{-1} P as $NaH_2PO_4.H_2O$, 20 mg L^{-1} K as K_2SO_4, 20 mg L^{-1} Ca as $CaCl_2.2H_2O$, 20 mg L^{-1} Mg as $MgSO_4.7H_2O$ and traces of Mn, B, Zn, Cu and Fe as described by Yoshida *et al.* (1976). After one week, healthy seedlings were transplanted into 2.5-L plastic pots, each containing 500 g of air-dried quartz sand with 0.1% (w/w) rice straw (to enhance reduction in the hypoxic media). There were 20 plants per pot. Half the pots had drainage holes and served as media for the aerobic treatments. The remaining pots without drainage holes allowed the sand to be flooded with 50 mm layer of nutrient solution. Nutrient solution pH was maintained at 5.6 throughout the experiment. Treatments were replicated three times and randomly arranged on laboratory tables.

The plants were grown at 26±1 °C with supplementary lighting which provided a photosynthetic flux density of about 1000-1200 mmol m^{-2} s^{-1} and a 16 h photoperiod. Oxidation potential (Eh) readings in all media were made twice daily (at 0900 and 1600 h) by inserting bright Pt electrodes (2 per pot) and a calomel reference electrode 80 mm deep into the sand.

Measurement of radial oxygen loss (ROL)

The Ti^{3+}-citrate solution was prepared under N_2 atmosphere (to prevent exposure of the solution to air) according to the method of Zehnder and Wuhrmann (1976). Three hundred mL of deoxygenated water was added to 17.647 g of sodium citrate to give 0.2 M sodium citrate solution. Thirty mL of 1.16M titanium chloride (Aldrich Chemical Co., Milwaukee, WI) was then added to the sodium citrate solution, and the pH adjusted to 5.6 by adding saturated sodium carbonate. The initial pH of freshly prepared Ti^{3+}-citrate buffer is about 2 with a low redox potential of <-200 mV. The rate of O_2 released through rice roots was measured at 14 and 35 d after transplanting. Forty mL of nutrient solution was poured into 50-mL test tubes and Ar gas bubbled through the solution for 1200 s to remove any dissolved O_2. Plant samples (one per test tube), previously washed carefully of any foreign matter and with the base of the plants (up to 6 cm above the collar) coated with Parafilm (American Can Co., Greenwich, CT), were inserted into the tubes. The roots were completely immersed in the nutrient solution. A 5-mL aliquot of Ti^{3+}-citrate was then injected into each test tube with a plastic syringe, followed immediately by layering the solution surface with 20 mm of paraffin oil to hinder atmospheric O_2 contamination. Plant samples were arranged in the test tubes such that the paraffin oil had no contact with roots. Control treatments had no plants. All the test-tubes with plants were kept under the same laboratory conditions as the

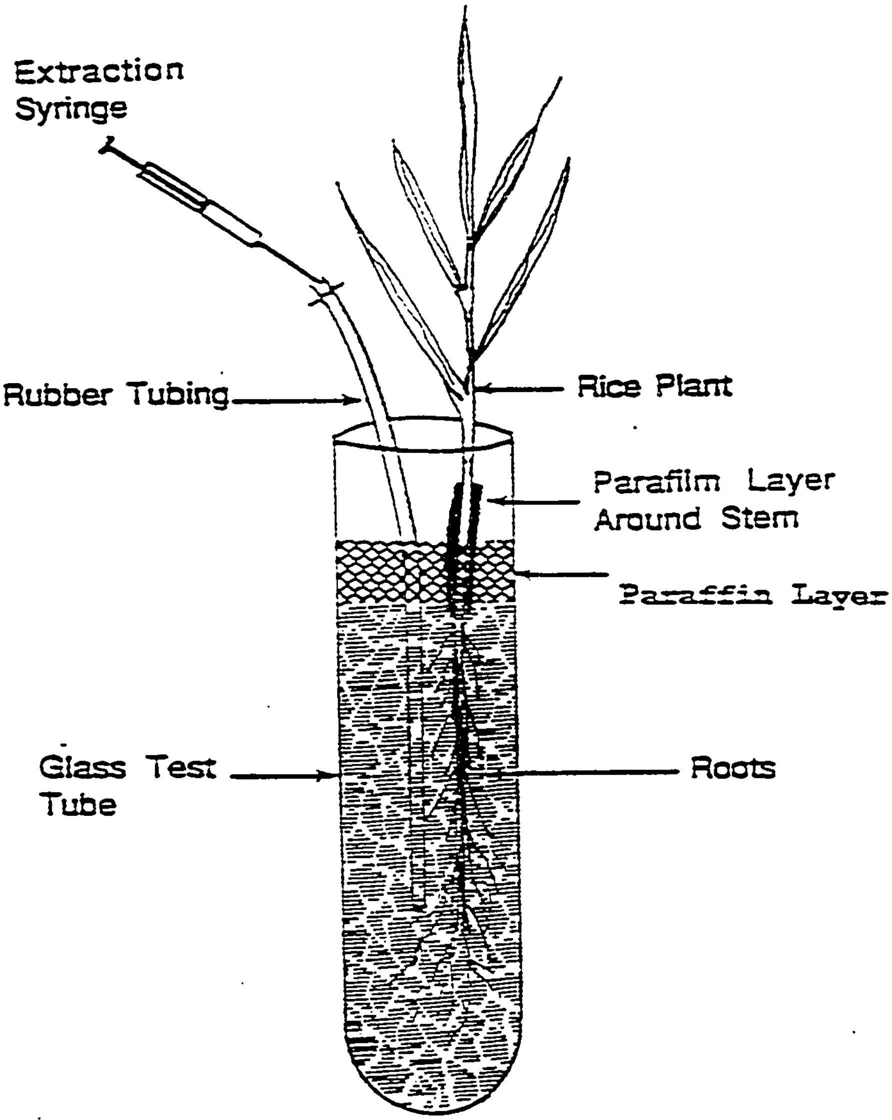

Figure 1.1: Experimental apparatus used to estimate radial oxygen loss from rice roots into titanium-citrate buffer solution.

experimental pots. After 6 h, the test-tubes were gently shaken and solution samples were taken with a syringe through rubber tubing that had been introduced into the solution alongside the roots (Fig. 1).

Absorbance of the partly oxidized Ti^{3+}-citrate solution was measured at 527 nm on a Perkin-Elmer 3 UV/VIS spectrophotometer. Released O_2 on whole plant basis was determined by extrapolation of the measured absorbance to a standard curve previously obtained from either of the following two calibration procedures:

(i) Using standards of known O_2 concentration. Series of ultra pure O_2 ranging from 5.0 to 200.0 μmol O_2 at standard temperature and pressure were introduced into Vacutainers (Becton Dickson, Lincoln Park, NJ) containing 970 μmol Ti^{3+} in the Ti^{3+}-citrate solution being used. The Vacutainers were previously evacuated. After manually shaking the gas-solution mixture for 60 s, the absorbance was determined. The equation relating the absorbance to the concentration of O_2 was $y=0.463-0.00145x$ ($r=0.96$). Net rate of ROL can be expressed in the following equation:

$$ROL = 4(a-b)$$

where ROL = radial oxygen loss, μmol O_2 $plant^{-1}$ d^{-1}.

a = oxygen concentration in the root solution after 6 h treatment with plants, μmol O_2 d^{-1}.

b = oxygen concentration in the solution without plant treatment (control), μmol O_2 d^{-1}.

(ii) Using dilution series of Ti^{3+} concentration in the Ti^{3+}-citrate solution. Dilution series were prepared from deoxygenated deionized water and a range of 10 to 200 μmol active Ti^{3+} of the Ti^{3+}-citrate solution being used. The series were prepared in evacuated Vacutainers under N_2 atmosphere and then measured for their absorbance. There was a significant linear relationship between absorbance of the solution and Ti^{3+} concentration, $y=0.12819+0.00015x$ ($r=0.98$) The range of variation in absorbance of samples at each Ti^{3+} concentration was less than 3% of the mean. Net rate of ROL was calculated as:

$$ROL = [c(y-z)]$$

where

ROL = radial oxygen loss, μmol O_2 plant^{-1} d^{-1}.

c = initial volume of Ti^{3+}-citrate added to each test tube, L.

y = concentration of Ti^{3+} in solution of control (without plants), μmol Ti^{3+} L^{-1}.

z =concentration of Ti^{3+} in solution after 6 h treatment with plants, μmol Ti^{3+} in solution plant^{-1} L^{-1}.

For comparison purposes, ROL was measured with a polarographic oxygen electrode, YSI model 51B oxygen meter (Yellow Springs instruments Co. Inc., Ohio, USA) and with the Ti^{3+} technique at plant age of 42 d. Plants samples of three of the rice cultivars, *Lemont, Rico and Mercury* (10 per method) were independently and randomly sampled from flooded pots and assigned to the two methods. Roots of intact plants were placed in a deoxygenated nutrient solution in air-tight 250-mL flasks. The oxygen electrode was calibrated in water. After 6 h, the oxygen probe was immersed in the solution to measure ROL directly.

Measurements of root porosity (Root air-space:POR).

After ROL measurements, roots were rinsed with deionized water. Root porosity was measured with a pycnometer (Jensen *et al.*, 1969). A 25-mL pycnometer was filled with water and weighed. About 0.2 to 0.3 g fresh roots were cut 2 to 2.5 cm from the apex of primary roots, blotted on dry tissue paper and weighed separately. The samples were introduced into the water-filled pycnometer and reweighed. The roots were later retrieved, ground into a paste with mortar and pestle, and returned to the pycnometer for reweighing. All precautions were taken to reduce loss of root materials as described in detail by Jensen *et al.*, (1969). Root porosity was determined with the formula:

$$POR = \frac{[(p\&gr)-(p\&r)]}{[(r+p)-(p\&r)]} \times 100$$

where POR = root air-space, %.

r = mass of fresh intact roots, g.

p = mass of water filled pycnometer, g.

p&r = mass of pycnometer with fresh intact roots and water, g.

p&gr = mass of pycnometer with ground roots and water, g.

Statistical analyses

Statistical analyses were performed on POR and ROL data collected on 5 plants randomly harvested from pots for each treatment, using PROC GLM and PROC CORR procedures available in SAS (1989). Significance of differences between the Ti^{3+}-citrate technique and the polarographic method was evaluated using 2-sample t-test with pooled variance (a=0.05). All POR values were square-root transformed prior to analysis. Data were also subjected to a 2-way analysis of variance, and mean separation with the Duncan's Multiple Range Test at the 0.05 level of probability. In cases where interactions existed between main effects, the Bonferonni method (Samuels, 1989) was used to determine differences among cultivars under the two soil regimes with an individual level of significance (Bonferroni Adjustment) of 0.0011 (general a level=0.05). The strength of linear relationship between POR (untransformed values) and ROL was assessed by determining correlation coefficients. Root porosity value of each sample was matched with its corresponding ROL value.

RESULTS AND DISCUSSION

Soil redox potential

Redox potential ranged between +580 and +620 mV in drained pots while those in flooded pots ranged from +330 to +340 mV and considered to be hypoxic. The relatively high Eh values in the media in spite of 0.1% rice straw application may be ascribed to low microbial populations and activity.

Under conditions of the experiment, there was no significant difference between the Ti^{3+}-citrate technique and the polarographic method of estimating O_2 loss from roots at 0.01% level of significance, as exemplified by data on *Lemont* (Table 1.1). Similar results were obtained for the other rice cultivars *Rico* and *Mercury*, and also for *Taxodium distichum* (Kludze *et al.*, in press). These results demonstrate that the Ti^{3+}-citrate technique provides sufficient accuracy for determining dissolved O_2 leaked from a whole root system. The fast rate of reaction of Ti^{3+} with O_2 indicates the sensitivity of the technique; the Ti^{3+} acts as a strong O_2 sink thus maintaining an O_2 concentration gradient between the roots and the Ti^{3+}-citrate solution. The titanium-citrate buffer also provides a good analogue of natural reduced soils because the Ti^{3+} ions mimic the O_2 sink of such soils by reacting with O_2 as it is released by roots, thus preventing it from being reabsorbed by root respiration. In addition, problems and errors normally associated with other colorimetric techniques were mitigated. For example, errors due to atmospheric air contamination of samples were reduced by including control samples (Ti^{3+}-citrate samples without plant treatments) in the computation of ROL. Since seedlings of all cultivars tested in this study were at the same age, the observed differences were assumed to be genetic and not due to defects in the technique.

Root porosity in response to soil aeration status.

Root porosity, a reflection of aerenchyma formation, occurred in plants under both drained and flooded conditions, and seedling age further enhanced this

Table 1.1: T-test comparing the Ti^{3+}-citrate technique with a polarographic method of estimating dissolved oxygen in solution (n=10). Values are for the cultivar, Lemont.

Method	Mean[1]	SD	T	P>ITI
Ti^{3+}-citrate	25.42	1.78	0.42	0.679
Polarographic	24.98	2.78	0.42	0.679

[1] Values of O_2 concentration were expressed in μmol O_2 plant^{-1}·d^{-1}.

Table 1.2: Mean values (n=5) for root porosity (POR) of seven rice cultivars at 14 and 35 days after transplanting in nutrient fertilized sand culture under drained and flooded conditions.

Cultivar	Treatment			
	Drained		Flooded	
	%			
	14 d	35 d	14 d	35 d
Rico	13.5	16.5	20.0	27.9
Lemont	13.7	17.0	20.5	23.0
Mercury	13.1	15.3	15.8	19.4
Mars	13.2	14.8	16.0	19.2
Tebonnet	13.4	14.0	15.2	19.0
Millie	12.7	13.4	13.2	15.6
Katy	12.1	12.3	12.3	13.6

Table 1.3: Analysis of variance for root porosity (POR)[1] of seven rice cultivars at 14 and 35 days after transplanting in nutrient fertilized sand culture under drained and fertilized conditions (n=5).

		Mean squares	
Source of variation	df	14 d	35 d
		%	
Drainage status (D)	1	0.0138	0.931**
Cultivar (C)	6	0.1000**	0.888**
DxC	6	0.0068	0.0436*

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[1] Values were square-root transformed.

*, ** Significant at the 0.5 and 0.1 levels of probability.

parameter especially for flooded treatments (Table 1.2). Drainage status (D) and cultivar (C) factors interacted (DxC) significantly to influence root porosity at 35 days in the flooded treatments (Table 1.3). Our findings are therefore consistent with the earlier work of Armstrong (1971) but partially contradict the reports of John *et al.* (1974) and Jackson *et al.* (1985). Although air space was measured even in plants under well-drained conditions, hypoxia stimulated its further formation. This may imply that although aerenchyma formation in rice could be a genetic trait that expresses itself spontaneously, O_2 stress enhances it. Such a phenomenon has also been reported by Laan *et al.* (1989) in some *Rumex* species. Under more reducing conditions than those obtained in this study, it is possible that there could be even more root air-space formation. Additional studies are needed to evaluate the response of rice cultivars to a wide range of soil oxidation potentials.

Radial oxygen loss (ROL) from rice roots in response to soil aeration status.

More O_2 was released from plant roots on day 35 than on day 14 under both drained and flooded conditions (Table 1.4). Oxygen release also depended significantly on both drainage status (D) and cultivar (C) and also on the interaction of these two factors (DxC) (Table 1.5). The highly significant DxC interaction at both sampling times means that the relative responses of the cultivars in terms of ROL are different, depending on the aeration status of the sand culture. On day 14 under drained conditions, the cultivars *Rico, Lemont* and *Mercury* did not significantly differ from each other, but differed from the other cultivars ($p<0.5$). Similarly, under flooded conditions, *Rico* and *Lemont* differed from the other cultivars, but did not differ from each other. Oxygen leakage from roots ranged from between 10.4 μmol plant^{-1} d^{-1} in *Katy* to 19.3 μmol plant^{-1} d^{-1} in *Rico*. A similar pattern was observed in treatments on day 35. By comparison, *Rico, Lemont* and *Mercury* released significantly more O_2 than the other cultivars under both drained

Table 1.4: Mean values (n=5) for radial oxygen loss (ROL) from roots of seven rice cultivars at 14 and 35 days after transplanting in nutrient fertilized sand culture under drained and flooded conditions.

	Treatments			
	Drained		Flooded	
	μmol O_2 plant^{-1} d^{-1}			
	14 d	35 d	14 d	35 d
Rico	14.0	18.2	19.3	33.2
Lemont	13.2	18.6	18.0	25.0
Mercury	13.4	16.2	16.3	24.7
Mars	11.0	15.0	15.3	19.2
Tebonnet	12.0	16.2	16.8	20.0
Millie	10.2	14.7	10.9	21.0
Katy	10.0	14.0	10.4	17.4

and flooded treatments.. All the cultivars, except *Katy* and *Mars*, released more than 20.0 μmol O_2 plant^{-1} d^{-1} under hypoxic conditions.

Apparently more O_2 leaked from the more efficient 'oxygen-conducting' cultivars because of higher correlation between root air space and O_2 leakage from those cultivars (Table 1.6). According to Luxmoore *et al.* (1972) and Armstrong (1979), the effectiveness of gas transport is dependent on two main factors: (i) the physical resistance to diffusion (which is directly proportional to root length and is inversely proportional to fractional root porosity, and (ii) O_2 demand along the diffusion path (which is a function of respiratory uptake and passive O_2 leakage from roots).

Differences in ROL could be related to root length, root mass or root surface area (Armstrong, 1979). Although these variables were not measured in the study, it is possible that they influenced the variability in ROL of the cultivars. Other factors that may influence O_2 loss from roots include the degree of cuticularization and suberization of the epidermal cells, constriction in the diffusion pathway at the stem base, and the level of interconnection between shoot aerenchyma and root aerenchyma.

The lower efficiency of some of the cultivars, especially *Mars*, in diffusing O_2 could presumably be ascribed to some or all these factors. This implies that possession of large aerenchyma system *per se* may not necessarily guarantee correspondingly high O_2 leakage from roots. It is pertinent to note that O_2 demands of roots and the rhizosphere are competitive (Armstrong and Beckett, 1987; Armstrong *et al.*, 1991). Cultivars with less respiratory activity would therefore be likely to release more O_2 than those with higher activity. It is therefore suggested that such root-rhizosphere competitive phenomenon may be a cause for the poor POR-ROL correlation in the affected cultivars. There is the need to investigate this further. Joshi *et al.* (1975) reported that varietal differences in tolerance to

Table 1.5: Analysis of variance for radial oxygen loss (ROL) from seven rice cultivars at 14 and 35 days after transplanting in nutrient fertilized sand culture under drained and flooded conditions (n=5).

		Mean squares	
Source of variation	df	14 d	35 d
		μmol O_2 plant^{-1}. d^{-1}	
Drainage status (D)	1	276.3**	1462.7**
Cultivar (C)	6	116.3**	200.9**
DxC	6	17.7**	11.9**

** Significant at the 0.01 level of probability.

Table 1.6: Linear correlation coefficient (r) relating radial oxygen loss (ROL) and porosity (POR) at 14 (a) and 35 (b) days after transplanting (n=5).

(a)

Cultivar	Treatment			
	Drained		Flooded	
	r	P>F	r	P>F
Rico	0.86	0.13	0.97	0.02*
Lemont	0.77	0.23	0.95	0.05*
Mercury	0.80	0.20	0.95	0.05*
Mars	0.81	0.20	0.78	0.23
Tebonnet	0.92	0.06	0.90	0.09
Millie	0.87	0.13	0.94	0.06
Katy	0.90	0.10	0.97	0.03*
(b)				
Rico	0.86	0.14	0.97	0.02*
Lemont	0.79	0.20	0.98	0.02*
Mercury	0.79	0.20	0.94	0.05*
Mars	0.81	0.19	0.60	0.36
Tebonnet	0.90	0.10	0.86	0.14
Millie	0.78	0.20	0.87	0.13
Katy	0.83	0.17	0.96	0.03*

* Significant at the 0.05 level of probability.

'straighthead' and 'mild sulfide' diseases (both are physiological disorders of the rice plant) could be attributed to differences in ROL. The high POR-ROL correlation in *Rico* and *Lemont* suggests the ability of these cultivars to tolerate reducing conditions and may explain why they are currently grown successfully in low-land rice cultivation on a larger scale in Louisiana.

REFERENCES TO CHAPTER 1

Armstrong, W. 1979. Aeration in higher plants. In: Advances in Botanical Research, vol. 7 (Ed. by H.W. Woolhouse) pp. 226-332. Academic Press, London, U.K.

Armstrong, W. 1971. Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration and waterlogging. Physiol. Plant. 25:192-198.

Armstrong, W. 1967. The use of polarography in the assay of oxygen diffusing from roots in anaerobic media. Physiol. Plant. 20:540-553.

Armstrong, W., and P.M. Beckett. 1987. Internal aeration and the development of stelar anoxia in submerged roots: A multi-shelled mathematical model combining axial diffusion of oxygen in the cortex with radial losses to the stele, the wall layers and the rhizosphere. New Phytol. 105:221-245.

Armstrong, W., P.M. Beckett, S.H.F.W. Justin, and S. Lythe. 1991. Modelling and other aspects of root aeration. In: Plant Life Under Oxygen Stress (eds. M.B. Jackson, D.D. Davies and H. Lambers), pp. 267-282. SPB Academic Publishing bv. The Hague.

Boon, P.I., and B.K. Sorrell. 1991. Biogeochemistry of billabong sediments. I. The effect of macrophytes. Freshwater Biol. 26:209-226.

DeLaune, R.D., S.R. Pezeshki, and J.H. Pardue. 1990. An oxidation-reduction buffer for evaluating the physiological response of plants to root oxygen stress. Environ. Exp. Bot. 30:243-247.

Glinski, J., and W. Stepniewski. 1983. Soil aeration and its role for plants. CRC Press, Inc., Boca Raton, Florida. pp. 173-188.

Jackson, M.B., M.F. Trevor, and W. Jenkins. 1985. Aerenchyma (gas-space) formation in adventitious roots of rice not controlled by ethylene or small partial pressures of oxygen. Planta 168:492-499.

Jenson, C.R., R.J. Luxmoor, S.D. Van Gundy, and L.H. Stolzy. 1969. Root air-space measurements by a pycnometer method. Agron. J. 61:474-475.

John, C.D., V. Limpinuntana, and H. Greenway. 1974. Adaptation of rice to anaerobiosis. Aust. J. Plant Physiol. 1:513-520.

Johnson, C.M., P.R. Stout., T.C. Broyer, and A.B. Carton. 1957. Comparative chlorine requirements of different plant species. Plant Soil 1:337-353.

Joshi, M.M., I.K.A. Ibrahim, and J.P. Hollis. 1975. Hydrogen sulfide: effects on the physiology of rice plants and relation to straighthead disease. Phytopathol. 65:1165-1170.

Justin, S.H.F.W., and W. Armstrong. 1987. The anatomical characteristics of roots and plant response to soil flooding. New Phytol. 106:465-495.

Kludze, H. K., S.R. Pezeshki, and R.D. DeLaune. Evaluation of root oxygenation and growth in baldcypress in response to short-term soil hypoxia. Can. J. For. Res. (in press).

Laan, P., A. Smolders, C.W.P.M. Blom, and W. Armstrong. 1989. The relative roles of internal aeration, radial oxygen losses, iron exclusion and nutrient balances in flood-tolerance of *Rumex* species. Acta Bot. Neerl. 38(2):131-145.

Luxmoore, R.J., R.E. Sojka, and L.H. Stolzy. 1972. Root porosity and growth responses of wheat to aeration and light intensity. Soil Sci. 113:354-357.

Pezeshki, S.R., S.W. Matthews, and R.D. DeLaune. 1991. Root cortex structure and metabolic responses of *Spartina patens* to soil redox conditions. Environ. Exp. Bot. 31:91-97.

Sale, P.J.M., and R.G. Wetzel. 1983. Growth and metabolism of *Typha* spps. in relation to cutting treatments. Aqua. Bot. 15:321-327.

Samuels, M.L. 1989. Statistics for the life sciences. Dellen publishing Co. San Francisco, CA.

SAS Institute Inc.1989. SAS/STAT User's Guide , Version 6, Fourth Edition, Vol. 2. SAS Institute Inc., Cary, North Carolina pp. 846

Smirnoff, N., and R.M.M. Crawford. 1983. Variation in the structure and response of flooding of root aerenchyma in some wetland plants. Ann. Bot. 51:237-249.

Taylor, G.J., A.A.Crowder, and R. Rodden. 1984. Formation and morphology of an iron plaque on the roots of *Typha datifolia* grown in solution culture. Am. J. Bot. 71:666-675.

Van Raalt, M.H. 1940. On the oxygen supply of rice roots. Ann. Tardin Bot. Buitenzong. 50:99-113.

Winkler, L.W. 1888. Die Bestimmung des in Wasser gelosten Sauerstoffs. Ber.Dtsch. Chem. Ges. 21:2843-2854.

Yoshida, S., D.A. Fomo, J.H. Cock, and K.A. Gomez. 1976. Laboratory manual for physiological studies of rice. Int. Rice Res. Inst., Los Banos, Philippines.

Zehnder, A.J.B., and K. Wuhrmann. 1976. Titanium(III) citrate as a non-toxic oxidation reduction buffering system for the culture of obligate anaerobes. Science 194:1165-1166.

CHAPTER 2

INTERACTIVE EFFECTS OF CULTIVAR TYPE AND STRAW APPLICATION RATE ON GROWTH, ROOT POROSITY AND GAS TRANSPORT IN RICE

INTRODUCTION

Exogenous organic matter application is one of the management practices adopted in wetland rice cultivation. Rice straw stubble is often left on the field from previous growing season and plowed into paddy soils prior to flooding, to provide nutrients to growing plants (Takahashi, 1964; Wells and Turner, 1984). The ready release of NH_4^+-N from anaerobically decomposing organic materials is partially responsible for the good response of rice to added straw (Patrick *et al.*, 1985). Straw has also been shown to markedly increase nitrogen fixation (Matsuguchi, 1979) and benefit K and S deficient soils (Ponnamperuma, 1984). The current emphasis on sustainable agriculture and energy conservation may bring about increased use of organic nitrogen in rice production (Sass *et al* ., 1991).

Application of straw to paddy fields, whether or not in combination with mineral fertilizers, has generally been found to enhance CH_4 emissions (Schutz *et al.*, 1989; Nouchi *et al.*, 1990; Yagi and Minami, 1990). In recent years, CH_4 emissions have been received much attention because of its contribution to the "greenhouse warming effect" of the troposphere. The concentration of atmospheric CH_4 showed a 1.0 % annual increase in the last decade (Rasmussen and Khalil, 1986; Khalil and Rasmussen, 1989). Paddy fields are estimated to contribute 70-170 Tg yr^{-1} of the total emissions, 375-717 Tg yr^{-1} (Schutz *et al.*, 1989). Yagi and Minami (1990) reported that straw applications at the rates of 6 ton ha^{-1} and 9 ton ha^{-1} increased CH_4 emissions by a factor of 3.3 and 3.5, respectively. Application rate of 12 ton ha^{-1} was however reported to decrease CH_4 emissions (Yagi and Minami, 1990) reported decreasing . It was suggested (1990; Bouwman, 1991) that these decreases were due to inhibition of methanogenesis by fermentative products of organic matter decomposition. The effects of the fermentative products on plant morphology and physiological functions, such as root development and air-space formation, that could influence the diffusive pathway of CH_4 have however been overlooked by researchers. The rice plant may bee the main route of CH_4 fluxes to the atmosphere (Cicerone and Shetter, 1981; Seiler *et al.*, 1984), accounting for more than 90 % of the total fluxes from paddy soils (Inubishi *et al.*, 1989).

An important plant-bacteria interaction in a flooded soil/sediment is the transport of gases between the above-ground photosynthesizing parts of wetland plants and the root system via intercellular spaces and aerenchyma (Armstrong, 1979; Dacey and Klug, 1979; Armstrong and Armstrong, 1988). Increases in formation of volatile fatty acids (VTA) and ethylene as a result of organic matter application to paddy fields have been reported to induce anatomical, physiological and morphological modifications, including aerenchyma formation, in plants (Lynch, 1978; Drew *et al.*,1979; Jackson and Drew, 1984; Justin & Armstrong, 1987). The aerenchyma enhances exchange of gases (e.g. O_2 and CH_4) between the root rhizosphere and the atmosphere (Armstrong and Webb 1985; Nouchi *et al.*, 1990). This gas transport can therefore affect CH_4 emissions to the atmosphere by regulating CH_4 oxidation in the rhizosphere through plant mediated changes in O_2 transport to the rhizosphere, and by regulating CH_4 emissions from the soil/sediment to the atmosphere through changes in resistance to CH_4 transport through the plant. Thus, diel and seasonal variations in observed CH_4 emissions may be correlated with observed variations in air movement through plants due to changes in factors such as plant photosynthetic activity, growth stage, cultivar type and other physiological variables. In addition, differences in observed CH_4 emissions may be correlated with variations in factors which affect root biomass and distribution such as cultivar type, soil properties and water management.

However, there is a paucity of information on cultivar and plant-related factors that control net CH_4 emissions. Neither is there any direct evidence for the cause of reduction in CH_4 emissions when high doses of organic matter are applied to paddy fields as reported by Yagi and Minami (1990) and Bouwman (1991). It is hypothesized here that interactions between rice straw application rates and rice cultivar type could cause differences in net amounts of CH_4 emitted as a result of the effects of these two variables, cultivar and straw application rates, on plant physiological functions. The objective of this study was therefore to determine the extent to which the interactive effects of rice cultivar type and dosages of rice straw can influence root porosity, plant growth and oxygen transport which in turn could influence net CH_4 emissions.

MATERIALS AND METHODS

Plant material

Seeds of three rice cultivars, *Katy* (long grain), *Mars* and *Rico* (medium grain) were soaked in 0.1% $HgCl_2$ for 60 s, rinsed with tap-water and germinated and nursed in nutrient solution, as described in Chapter 1. Two weeks after seed germination, healthy and uniform seedlings were selectively transplanted into plastic pots (120 mm i.d., 190mm effective height [e.h.]), each containing 1800g of Crowley silt loam.

Treatment with rice straw

The plow layer of Crowley silt loam (Typic Albaqualf) was collected from the Rice Research Station, Crowley, Louisiana. It was air-dried and ground to pass a 1.00mm mesh sieve. The soil had the following chemical properties: pH of 5.7 (1:1, ssoil/water ratio), organic matter content of 15.7 g kg^{-1}, C.E.C. of 9.4 $cmol_c$ kg^{-1}, 110.0 g kg^{-1} clay, and total C and N content of 7.0 and 0.5 g kg^{-1}, respectively.

Two levels of pulverized rice straw, 0.5% and 1.0% (w/w), equivalent to 11 and 22 tons ha^{-1}, respectively, were thoroughly mixed with the soil to constitute treatments 2 and 3 respectively; soil treatment 1 had no supplementary (0%) rice straw. The straw had a C/N ratio of 29. The pots were continuously flooded with deionized water by maintaining 30mm of water above the soil surface, starting five days before transplanting. Nine healthy seedlings of about equal size and height from each of the three cultivars were transplanted into the pots at a spacing of 35 mm by 35 mm. The controls (fallow) had no plants. The planted and unplanted treatments were replicated thrice for each cultivar. Plants were grown in the laboratory under the following conditions: day and night temperatures of about 25$\pm$1 °C and 23$\pm$1 °C respectively, 18-h photoperiod with light intensity of 1000-1100 μmol m^{-2} s^{-1} at canopy level. Temperature in the pots was 28$\pm$2 °C

Measurements

Measurements of soil redox potential (Eh, in millivolts) were made every other day in duplicate, using Pt electrode (permanently installed in the pots), saturated calomel reference electrode and Orion Research Model 231 Digital mV meter. The standard potential of the reference electrode (+245 mV), was added to each reading to obtain the Eh of the soil medium. Soil pH measurements were made at the same time with combine pH electrode.

A modified closed chamber method, as described by Minami and Yagi (1988), was used to measure CH_4 fluxes. The chambers were constructed from plexiglas cylinders (i.d. 100mm, e.h. 440mm). Each chamber had a fan fixed on its upper wall for homogenizing the air prior to gas sampling. The chamber was mounted on top of the plastic pots enclosing all the 9 rice plants, using a plastic plate as a base between the edges of the pot and the gas chamber. This set-up was sealed air-tight with non-toxic RTV Rubber Sealant (General Electric, Waterford, NY). Methane flux was determined by measuring the temporal increase of CH_4

concentration of air within the chamber. Air sampling was performed immediately after mounting the chamber and after every one hour for 5 h.

Concentrations of gas collected from each pot were determined with a Perkin-Elmer 200 gas chromatograph equipped with a flame ionization detector (Lindau *et al.*, 1991). Methane standards containing 10.5 and 110 μL CH_4 L^{-1} air were used to construct a standard curve from which the amounts of CH_4 in the gas samples were determined. Rates of CH_4 emission were determined by regression analysis of CH_4 accumulation as a function of time by using a modified equation of Rolston (1986):

$$F = (v/a)/(\Delta c/\Delta t)$$

where F= methane emission rate

v= volume of headspace of gas collecting chamber

a= soil area

$\Delta c/\Delta t$ = change of CH_4 gas concentration, unit $time^{-1}$.

Methane emission measurements were determined at 20, 30 and 40 d after transplanting. Emission rates were expressed in mg CH_4 m^{-2} d^{-1}.

As it was practically difficult to harvest plants without losing both soil and root materials whilst the experiment progressed, ROL and POR measurements were done only at the end of the experiment (40 d after transplanting) as described in detail in Chapter 1. There were 5 replicates for the ROL and POR measurements. Root and shoot dry weights and root length were subsequently determined.

Statistical analyses

The experiment was conducted in a completely randomized design with a split-plot arrangement of factorial treatments. Factorial combinations of cultivar type and straw dosage represented the main-plot factor and sampling time the sub-plot factor. After the initial randomization scheme, pots were rearranged on laboratory benches every three days to minimize positional effects and reduce bias in the estimation of the treatment means. Data on variables measured (methane emissions, POR, ROL and plant growth parameters) were analyzed using the PROC GLM procedure available in Statistical Analysis System (SAS Institute Inc., 1989). Regression analyses were performed to assess the relationship between ROL and POR. In cases where interactions existed between main effects, the Bonferroni method (Samuels, 1989), was applied as explained in Chapter 1.

RESULTS AND DISCUSSION

Soil redox changes

Soil redox potential (Eh), a measure of the reduction intensity of the soil, decreased rapidly with time. By day 15, soil Eh reached values of -130 mV and -240±20 mV in the straw untreated and treated pots, respectively. The low Eh readings in the straw treated soils remained fairly constant up to 35 d after which the Eh began to rise in the treatments with 0.5 % straw. In the untreated soil, soil Eh began to increase by day 25, indicating the exhaustion of carbon substrate supply. Soil pH ranged between 6.9 and 7.1 in the straw treated soils and between 6.7 and 6.8 in the untreated soils. There were no significant variations in Eh and pH measurements between planted and unplanted pots, indicating that vegetation did not have much influence on those soil factors within the experimental period of the study.

Plant growth

Rice growth, as represented by root and shoot weights, and root length were significantly ($p<0.05$) affected by both cultivar type and straw application rates. (Table 2.1) The interactive effects of the two factors were also significant, suggesting that the relative responses of cultivars in terms of growth are different, depending on the organic substrate level of the soil. In general, all the growth parameters in the three cultivars decreased with increase in straw application rates. In

Table 2.1: Effects of cultivar type and rice straw application rate on the growth of rice.

Factor	Root dry weight	Shoot dry weight	Root length
	g plant^{-1}	g plant^{-1}	cm
Cultivar (C)			
Katy	0.80b[1]	1.34c	11.8c
Mars	0.92ab	1.80b	12.1b
Rico	1.13a	2.10a	14.1a
Rice straw dosage (S)			
0.0%	1.43a	3.03a	15.4a
0.5%	1.13b	1.67b	13.1b
1.0%	0.32c	0.54c	9.5c
Cultivar(C) x Straw (S)			
Katy x 0.0%	1.3a	2.3a	14.1a
Katy x 0.5%	1.0a	1.5b	13.0a
Katy x 1.0%	0.2b	0.2c	9.0b
Mars x 0.0%	1.4a	3.2a	15.9a
Mars x 0.5%	1.1a	1.6b	11.0b
Mars x 1.0%	0.3b	0.6c	8.5c
Rico x 0.0%	1.6a	3.6a	16.1a
Rico x 0.5%	1.3a	1.9b	15.3a
Rico x 1.0%	0.5b	0.8c	10.9b
F-test for interactions			
Cultivar(C) x Straw (S)	*	*	*

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[1] Values followed by the same letter are not significantly different ($p<0.05$) Duncan Multiple Range Test.

* Significant at $p<0.05$

this respect, *Katy* was the most adversely affected cultivar as its root length and root and shoot dry weights were lower than those of the other two cultivars.

Inhibition of root and shoot growth in response to soil oxygen deficiency has been reported for many vascular plants under a variety of experimental conditions (Bradford and Yang, 1981; Cobb and Kennedy, 1987; Drew and Lynch, 1980). The incorporation of organic matter such as rice straw promotes the production and accumulation of organic acids in submerged soils (Gotoh and Onikura, 1971). These acids are toxic to rice at very low concentrations (Takijuma, 1963; Sass *et al.*, 1991) and may lead to reduced root growth and root decay (Kawata and Ishihara, 1961; Lynch, 1978). Although there was no complete death, plants grown under the maximum straw application rate (1.0 %) were stunted with necrosis of leaf tips and margins. These symptoms indicated a limitation in the movement of nutrients and water into the plant. Total root dry weight ranged from 15 to 31 % compared to plants without supplementary straw application. Under conditions of high organic matter application in a flooded soil, ethylene gas produced by both microbial activity and plant roots accumulates and inhibits root growth (Jackson, 1982). Straw application did not affect shoot dry weight to the same degree as they affected root dry weight. Consequently, shoot/root dry weight ratio increased with increase in straw application. However, the ratio varied among cultivars and also in the cultivar-straw interactions, indicating differences in response to soil conditions by cultivars.

Root porosity (POR)

Root porosity (POR), significantly varied ($p>0.05$) among cultivars (Table 2.2). Although there was no significant enhancement of POR when straw dosage was increased from 0.5 % to 1.0 %, straw application appeared to enhance POR compared to the untreated ones. The interactive effects of cultivar type and straw application rates were insignificant. In general, *Rico* had the highest aerenchyma

Table 2.2: Effects of cultivar type and rice straw application rate on root porosity (POR) and radial oxygen loss (ROL).

Factor	POR (%)	ROL (μmol plant^{-1} d^{-1})	CORR[1] P>F
Cultivar (C)			
Katy	23.7c[2]	19.5c	0.04*
Mars	31.7b	21.3b	0.18NS
Rico	39.2a	35.2a	0.03*
Rice straw dosage (S)			
0.0%	26.5b	22.2b	0.05*
0.5%	33.0a	28.1a	0.02*
1.0%	35.0a	16.7c	0.34NS
Cultivar (C) x Straw (S)			
Katy x 0.0%	20.0b	19.2a	0.03*
Katy x 0.5%	24.0ab	22.0a	0.04*
Katy x 1.0%	27.0a	14.3b	0.28NS
Mars x 0.0%	28.0a	21.3b	0.07NS
Mars x 0.5%	33.0a	29.1a	0.11NS
Mars x 1.0%	34.0a	16.5c	0.28NS
Rico x 0.0%	31.7b	35.2a	0.01*
Rico x 0.5%	42.0a	39.3a	0.02*
Rico x 1.0%	44.0a	19.2b	0.36NS
F-test for interactions			
Cultivar x Straw	NS[3]	**	

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[1] Linear correlation (r) relating ROL to POR.

[2] Values followed by the same letter are not significantly different ($p<0.05$) Duncan Multiple Range Test.

[3] NS=non significant F ratio ($p<0.05$); ** significant at $p<0.01$

formation while *Katy* had the least. Values ranged between 21 % in *Katy* at 11 ton ha^{-1} and 44 % in *Rico* at 22 ton ha^{-1}.

One way by which aquatic macrophytes enhance emission of gases involves the formation of large internal gas spaces to allow gas exchange between the reducing organic-rich sediments in which they are rooted and the atmosphere (Chanton *et al.*, 1992). Our study showed enhancement of gas-space development with application of of straw. Since organic matter decomposition products constitute additional stress on the plant, the roots possibly responded by increasing cortical cell breakdown to generate more air-space. Root porosity, which may be an indication of root aerenchyma formation in some plant species, appears to result from the development of hypoxic conditions followed by enhanced synthesis and accumulation of ethylene gas (Drew *et al.*, 1979; Drew *et al.*, 1981; Jackson *et al.*, 1985). Addition of organic matter to soils stimulates formation of ethylene (Yoshida and Suzuki, 1975) which is implicated in aerenchyma formation in most plants, including rice (Atwell et al., 1988; Seliskar, 1988; Justin and Armstrong, 1991).

Radial oxygen loss (ROL)

Radial oxygen loss from roots (ROL), significantly differed among rice cultivars and straw application rates (Table 2.2). Oxygen leakage was highest in *Rico* and lowest in *Katy*. The interactive effect of cultivar and straw were also found to be significant, suggesting that radial oxygen loss from roots of rice cultivars differs depending on the organic matter status of the soil. This is because organic matter modifies the morphology and physiology of plants which could in turn influence gas transport. In general, ROL was highest in plants that were grown in soils amended with 0.5% straw; doubling application rates to 1.0% drastically reduced ROL on per plant basis in all the cultivars. The effect was most astounding in *Rico* where ROL was curtailed by half (from 32.3.2 to 15.2 μmol $plant^{-1}$ d^{-1}). This phenomenon was most likely to be directly related to differences in root density

Table 2.3: Analysis of Variance for methane emissions as affected by rice cultivar, rice straw application rate and sampling time.

Source of Variation	df	Mean Squares[1]
Cultivar (C)	2	4372**
Straw dosage (S)	2	37719**
C x S	4	1345**
R (C x S)	18	14 NS
Sampling Time (T)	2	1563**
C x T	4	1210**
S x T	4	1219**
C x S x T	8	589**

[1] ** Significant at $p<0.05$; NS= not significant at $p<0.05$.

since at 1.0 % rice straw application rate, root weight and hence total pathway for gas transport decreased (Table 2.1). The concentration of Fe and Mn on root coatings (quantitatively determined by oxalic acid extraction) was highest in the treatments with the 1.0 % straw application rate, indicating high rates of oxidation of Fe^{2+} and Mn^{2+} ions on the root rhizoplane. It is suggested that such coatings could impede gas diffusion and may partly explain why both gas transport (O_2 leakage from roots to the rhizosphere and CH_4 diffusion from the soil into the plant) was inhibited at the high straw application rate (1.0 %). The effect of root coatings on the diffusion and transport of gases between the bulk soil and the air-spaces of plant roots needs to be investigated.

Linear correlation between ROL and POR within the experimental period (Table 2.2), indicated that cultivar-straw interactions induced significant and positive POR-ROL correlation in *Katy* and *Rico* at no straw (0.0 %) and 0.5 % straw treatments. *Mars* showed no correlation between POR and ROL. This trend is similar to results obtained in Chapter 1 and reaffirms the common knowledge that rice cultivars respond differently to soil conditions.

Methane emission rates

The Analysis of variance on CH_4 emissions is presented on Table 2.3. Cultivar, straw application rate and sampling time, together with their interactions significantly affected net CH_4 emissions. This three-way interaction suggests that the individual effect of these variables on CH_4 flux in rice cultivar cannot be easily separated. This is consistent with the findings of Sass *et al.* (1991). Figures 2a,b,c show the time course of CH_4 emissions. There were clear cut differences in emission rates between vegetated and fallow (unvegetated) treatments at all sampling times. Vegetated pots emitted more CH_4 than fallow pots at 0.0 and 0.5% straw application rate. Emissions were highest at 0.5% straw application rate for all the cultivars at all gas sampling times, ranging from 86 mg m^{-2} d^{-1} in *Katy* 20 d after transplanting, to

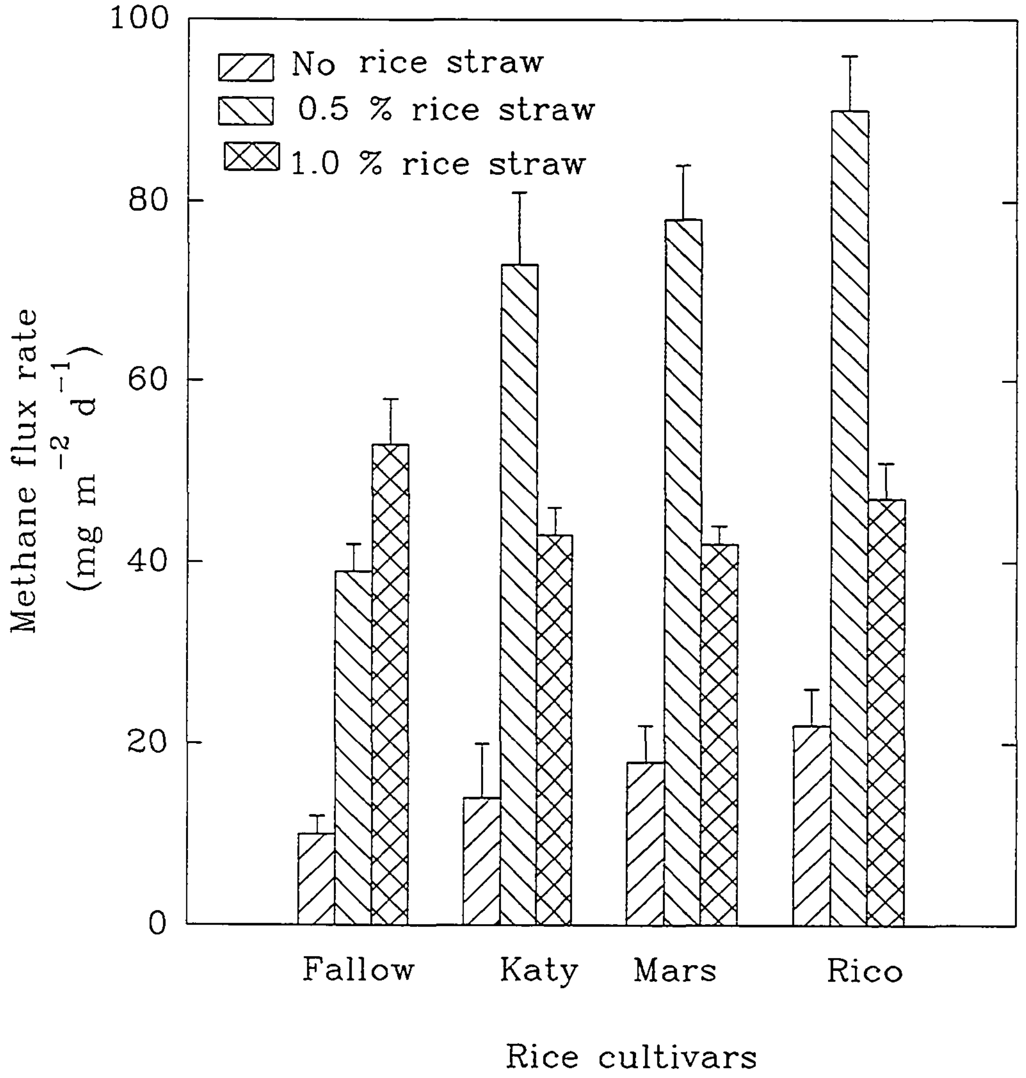

Figure 2.1a: Effect of rice straw application rates on methane emissions at 20 days after transplanting.

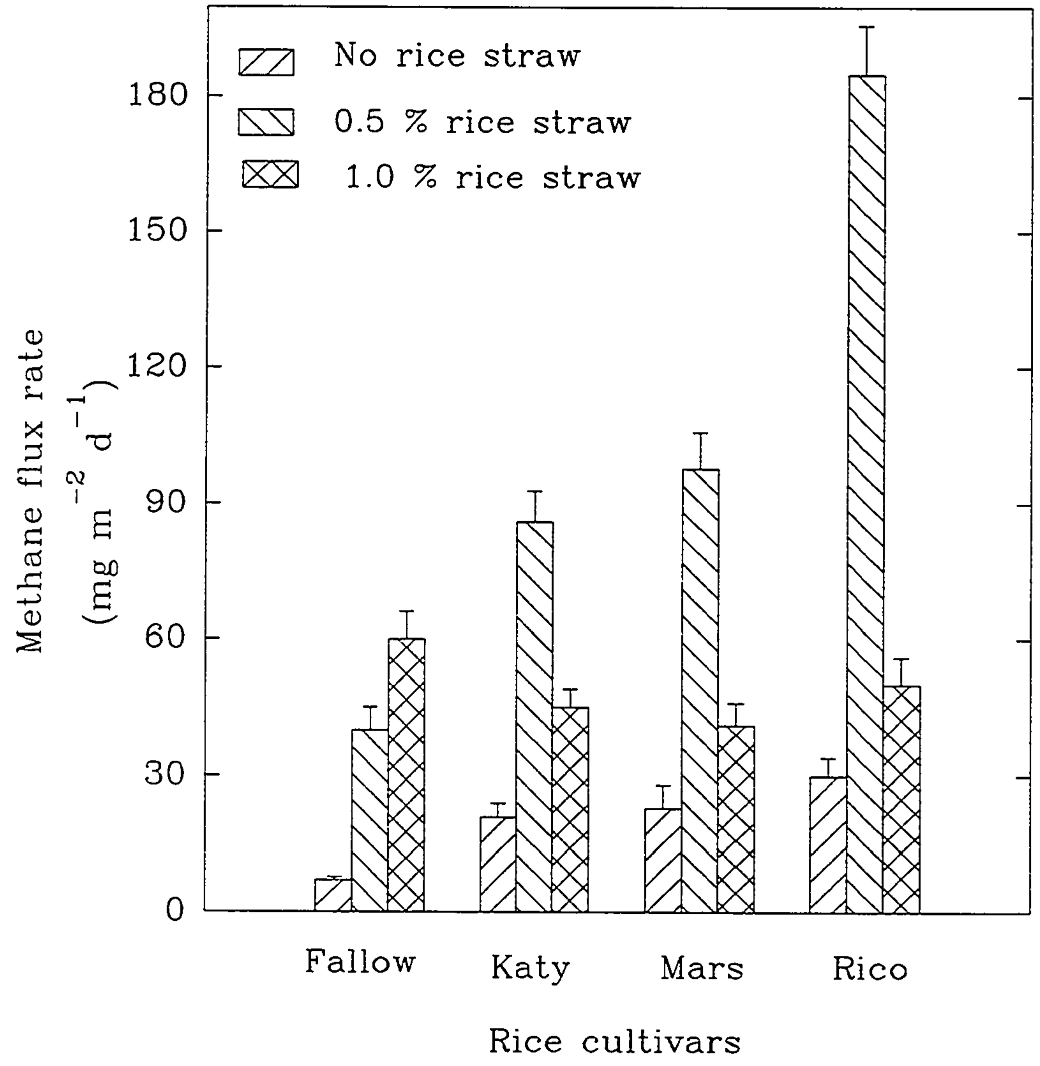

Figure 2.1b: Effect of rice straw application rates on methane emissions at 30 days after transplanting.

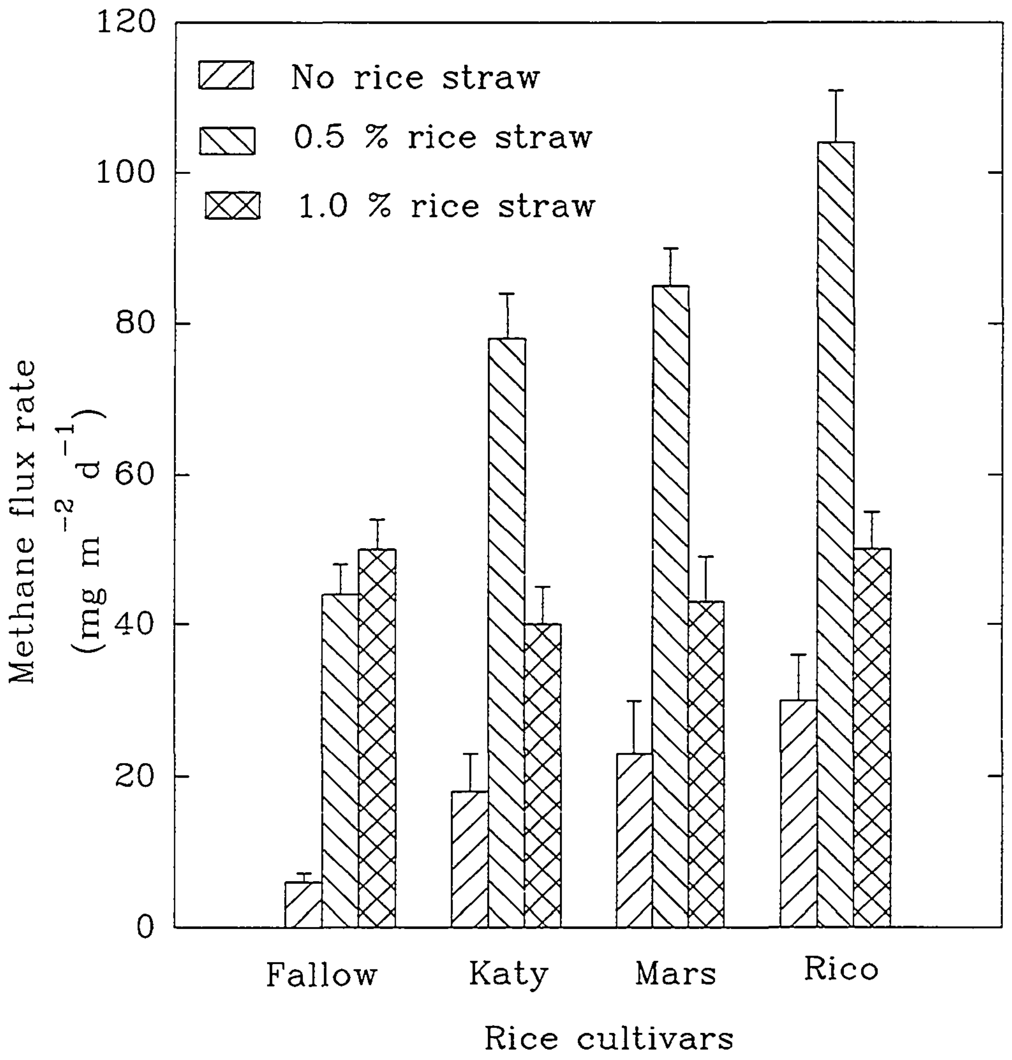

Figure 2.1c: Effect of rice straw application rates on methane emissions at 40 days after transplanting.

193 mg m^{-2} d^{-1} in *Rico* after 40 d of growth. At 1.0% rice straw application rate, plant mediated emissions decreased with time in all the cultivars. In treatments without straw application, CH_4 emission remained at very low levels compared with emissions from the treated pots, indicating that enhancement of methanogenesis could be effected in the Crowley soil only by supplementary organic substrate application.

In the unvegetated pots, rates of CH_4 emission declined with time at 0.0 and 0.5% straw application rates. Emissions however increased with time at 1.0% application rate, thus re-emphasising the importance of organic substrate availability for methanogenesis, and also suggesting that methanogenesis was not inhibited at this straw application rate. Evidence of increases in CH_4 emissions with increase in straw application rate from fallow (unplanted) pots was shown by a corresponding increase in bubble formation. Variations existed among cultivars in CH_4 emissions. *Rico* emitted the highest amount of CH_4 at all the three sampling times; emissions dramatically increased from 106 mg m^{-2} d^{-1} on day 30 to 193 mg m^{-2} d^{-1} 10 d later at 0.5% application rate. *Katy* emitted the least amount of CH_4 at all times. Emissions from *Mars* were between those of *Katy* and *Rico*, with the highest rate occuring on day 40 at 0.5% straw applicatin rate.

Methane is the final product of the anaerobic decomposition of organic matter. In general results of this study indicated that the highest emission rates were obtained at 0.5 % straw application rate and not at 1.0 % rate although CH_4 production was uninhibited in the Crowley soil at the 1.0 % rate (unpublished data; Wang *et al.*, 1992). Wang *et al.* (1992), found a positive correlation between CH_4 emission rate and straw application rate in Crowley soil up to a rate of 2.0 % (44 tons ha^{-1}). On the other hand, since plant biomass (root and shoot weights) was significantly reduced at 1.0 % straw application rate (Table 2.1), the reduction in the total diffusive pathway of the gas should be responsible for the lower emissions at 1.0% application rates. Whiting *et al.* (1991) and Sass *et al.* (1990) have reported a linear relationship between plant biomass and CH_4 emissions. Therefore, at straw application rates of up to 22 ton ha^{-1}, CH_4 emissions may not be reduced by methanogenesis as suggested by some researchers (Yagi and Minami 1990; Bouwman, 1991), but rather by the curtailment of the diffusive pathway of the gas through reductions in total plant biomass, particularly root density, and other plant related factors such as the mechanical impedence of root coatings. Studies by those researchers indicated reductions in CH_4 emissions without actually measuring CH_4 production rates; neither did they take into acccount plant-related effects on gas transport. It must however be noted that methanogenesis could be inhibited in acid soils that receive straw application at the rate of 22 tons ha^{-1} and above due to decreases in soil pH resulting from acidic decomposition products. Even under such a situation in the field, seepage and percolation could remove the acidic products beyond the sub-soil (Kimura *et al.*, 1992; Murase *et al.*, 1993).

Rice plants can enhance CH_4 flux by providing substrates for methanogenic bacteria through the production of root litter and root exudates (Holzapfel-Pschorn *et al.*, 1986; Sass *et al.*, 1990) that contain carbohydrates and amino acids. These nutrients stimulate microbial activity and fuel CH_4 production (Rovira, 1969; Schutz *et al.*, 1989). According to Vermoesen *et al.*, (1991), there is a strong correlation between water soluble carbon and CH4 production. The extra carbon from root litter and exudates may explain the disparity in CH4 emission between the vegetated and unvegetated pots that received no straw application. Also, differences existed among cultivars in CH_4 emissions from the no-straw treatments possibly because of diffences in their rate of root litter accumulation and root exudation which affect CH_4 production.

In conclusion, the results of this study suggest that the effect of incorporated rice straw on CH_4 emission is more complex than simple substrate addition to the soil. There was an interactive relationship between rice cultivar and rice straw application rates that influenced methanogenesis and root morphological and physiological changes (e.g porosity), which consequently influenced gas exchange between the rhizosphere and the atmosphere. The results thus validate the hypothesis of this study. Compared to results in Chapter 1, both root porosity (POR) and radial oxygen loss (ROL) from plants were increased when plants were grown in the soil characterized by greater O_2 stress. This indicates that the substrate condition in which food-tolerant plant species are grown need to be well defined in evaluating plant responses. Application of straw at 0.5% and 1.0% did not significantly affect changes in the bulk soil Eh. At these straw application rates, CH_4 emission was more dependent on the total pathway for gas escape to the atmosphere via the root aerenchyma. Whereas 0.5 % application rate enhanced gas transport (both CH_4 emission and radial oxygen loss), doubling the rate to 1.0 % reduced it plausibly because of reductions in plant biomass. Based on our results, it is evident that the cultural practice of rice straw application as organic fertilizer to paddy fields contribute to increasing emissions of CH_4 and could also adversely affect the growth of rice. A possible way of curbing CH_4 emissions through organic fertilizer application without sacrificing its beneficial effects may therefore be the reduction in application rates or use of composted materials as suggested by (Yagi and Minami, 1990). Organic materials with low C/N ratio, as found in composts, produce less CH_4. Cultivar selection is another way of mitigating CH_4 emissions. Investigation of the morphological and physiological differences in gas transport among cultivars that lead to different CH_4 fluxes should be intensified and should become part of breeding programs aimed at mitigating CH_4 emissions.

REFERENCES TO CHAPTER 2

Armstrong, J. 1979. Aeration in higher plants. In: Advances in Botanical Research, vol. 7 (Ed. by H.W. Woolhouse) pp. 226-332. Academic Press, London, U.K.

Armstrong, J., and W. Armstrong. 1988. *Phragmites australis*. preliminary study of soil oxidizing sites and internal gas transport pathways. New Phytol. 108, 373-382.

Armstrong, W., and T. Webb. 1985. A critical oxygen pressure for root extension in rice. J. Exp. Bot. 36: 1573-1582.

Atwell, B.J., M.C. Drew, and M.B. Jackson. 1988. The influence of oxygen efficiency on ethylene synthesis, 1-aminoclopropane-1-carboxilic acid levels and aerenchyma formation in roots of *Zea mays*. Physiol. Plant. 72:15-22.

Bouwman, A.F. 1991. Agronomic aspects of wetland rice cultivation and associated methane emissions. Biogeochem. 15:65-88.

Bradford, K.J., and S.F. Yang. 1981. Physiological responses of plants to waterlogging. Hort. Sci. 16:25-30.

Chanton, J.P.G., G.J.Whiting, W.J. Showers, and P.M. Crill. 1992. Methane flux from *Peltandra virginica*: Stable Isotope Tracing and Chamber effects. Global Biogeochem. Cycles 6(1):15-31.

Cicerone, R.J., and J.D. Shetter. 1981. Sources of atmospheric methane: Measurements in rice paddies and a discussion. J. Geophys. Res. 86:7201-7209.

Cobb, B.G., and R.A. Kennedy. 1987. Distribution of alcohol dehydrogenase in roots and shoots of rice (*Oryza sativa*) and *Echinochloa* seedlings. Pl. Cell Envir. 10:633-638.

Dacey, J.W.H., and M.J. Klug. 1979. Methane efflux from lake sediments through water lilies. Science 203:1253-1255.

Drew, M. C., M.B. Jackson, and S.C. Giffard. 1979. Ethylene- promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. Planta 147:83-88.

Drew, M. C., M.B. Jackson, S.C. Giffard, and R. Campbell. 1981. Inhibition of silver ions of gas space (aerenchyma) formation in adventitious roots of Zea mays L. subjected to exogenous ethylene or to oxygen deficiency. Planta 153:217-224.

Drew, M.C., and J.M. Lynch. 1980. Soil anaerobiosis, microorganisms and root functions. A Rev. Phytopathol. 8:37-66.

Gotoh, S., and Y. Onikura. 1971. Organic acids in flooded soil receiving added rice straw and their effect on the growth of rice. Soil Sci. Plant Nutr. 17:1-8.

Holzapfel-Pschorn, A., R. Conrad, and W. Sieler. 1986. Effects of vegetation on the emission of methane from submerged paddy soil. Plant Soil 92:223-233.

Inubishi, K., K. Hori, S. Matsumoto, M. Umebayashi, and H. Wada. 1989. Methane emission from flooded paddy soil to the atmosphere through rice plant. Jpn. J. Soil Sci. Plant Nutr. 60:318-324 (in Japanese with English summary).

Jackson, M.B. 1982. Ethylene as a growth promoting hormone under flooded conditions. In: Plant growth substances 1982. (Ed.) P.F. Wareing. Academic Press, London, pp. 291-301.

Jackson, M.B., and M.C. Drew. 1984. Effect of flooding on herbaceous plants. Flooding and Plant Growth (ED by T.T. Kozlowski), pp625-295. Academic Press, London.

Jackson, M.B., T. M. Fenning, and W. Jenkins. 1985. Aerenchyma (gas space) formation in adventitious roots of rice is not controlled by ethylene or small partial pressures of oxygen. Planta. 168:492-499.

Justin, S.H.F.W., and W. Armstrong. 1991.Evidence for the involvement of ethene in aerenchyma formation in adventitious roots of rice (*Oryza sativa* L.). New Phtol. 118:49-62.

Justin, S.H.F.W., and W. Armstrong. 1987. The anatomical characteristics of roots and plant response to soil flooding. New Phytol. 106:465-495.

Kawata, S., and K. Ishihara. 1961. Studies on the effects of some organic acids on the root hair formation in the root of rice plants. Nippon Sakumotsu Gakkai Kiji 30:72-78.

Khalil, M.A.K., and R.A. Rasmussen. 1989. Climate-induced feedbacks for global cycles of methane and nitrous oxide. Tellus. Ser. B, 41:554-559.

Kimura, M., Y. Miura, A. Watanabe, J. Murase, and S. Kuwatsuka. 1992. Methane production and its fate in paddy fields. I. Effects of rice straw application rate on the leaching of methane and other soil components into the subsoil. Soil Sci. Plant Nutr. 38:665-672.

Lynch, J.M. 1978. Production and phytotoxicity of acetic acid in anaerobic soils containing plant residues. Soil Biol. Biochem. 10:131-135.

Minami, K., and K. Yagi 1988. Method of measuring methane flux from rice paddies. Jpn. J. Soil Sci. Plant Nut. 59:458-463.

Matsuguchi, T. 1979. Factors affecting heterotrophic nitrogen fixation in submerged rice soils.In: IRRI, Nitrogen and Rice. (pp 207-221). International Rice Research Institute, Las Banos. Philippines.

Murase, J., M. Kimura, and S. Kuwatsuka. 1993. Methane production and its fate in paddy fields. III. Effects of percolation on methane flux distribution to the atmosphere and the subsoil. Soil Sci. Plant Nutr. 39:63-70.

Nouchi, I., S. Mariko, and K. Aoki. 1990. Mechanism of methane transport from the rhizosphere to the atmosphere through rice plants. Plant Physiol. 94:59-66.

Patrick, W.H. Jr., D.S. Mikkelson, Wells, B.R. 1985. Plant Nutrient Behaviour In Flooded Soil. Fertilizer Technology and Use (3rd Ed.) Soil Sci. Soc. Amer., 667 South Segoe Road, Madison, WI pp. 197-227.

Ponnamperuma, F.N. 1984. Straw as a source of nutrient for wetland rice soils. In: Organic matter and Rice, pp117-135. IRRI, Los Banos, Philippines.Rolston, D.E. 1986. Gas Flux. In Methods of soil analysis Part 1, Physical and mineralogical Methods. Ed A. Klute pp1103-1119.

Rasmussen, R.A., and M.A.L. Khalil. 1986. Atmospheric trace gases: Trends and distributions over the last decade. Science 232:1623-1624.

Rolston, D.E. 1986. Gas Flux. p.1103-1119. In A. Klute (ed.) Methods of soil analysis Part 1, Physical and Mineralogical Methods. ASA, Madison, WI.

Rovira, A.D. 1969. Plant root exudates. Bot. Rev. 35:35-57

Samuels, M.L. 1989. Statistics for the life sciences. Dellen publishing Co. San Francisco, CA.

SAS Institute Inc.1989. SAS/STAT User's Guide , Version 6, Fourth Edition, Vol. 2. SAS Institute Inc., Cary, North Carolina pp. 846.

Sass, R.L., F.M. Fisher, and P.A. Harcombe. 1991. Mitigation of methane emissions from rice fields: Possible adverse effects of incorporated rice straw. Global Biogeochem. Cycles 5:275-287.

Sass, R.L., F.M. Fisher, P.A. Harcombe, and F.T. Turner. 1990. Methane production and emission in agricultural wetlands. Global Biogeochem. Cycles 4:47-68.

Schutz, H., W. Sieler, and R. Conrad. 1989. Processes involved in formation and emission of methane in rice paddies. Biogeochem. 7:33-53.

Seiler, W., A. Holzapfel-Pschorn, R.Conrad, and D. Scharffe. 1984. Methane emission from rice paddies. J. Atmos. Chem. 1:241-268.

Seliskar, D.M. 1988. Waterlogging stress and ethylene production in the dune slack plant, *Scirpus americanus*. J. Expt. Bot. 39:1639-1648.

Takahashi, J. 1964. Natural supply of nutrients and plant requirements. In: the mineral nutrition of the rice plant, pp. 271-294. John Hopkins Press, Baltimore, Md.

Takijuma, Y. 1963. Studies on behavior of the growth inhibiting substances in paddy soils with special reference to the occurerence of root damage in the peaty paddy fields. Bull. Natl. Inst. Agric. Sci., Sec. B. 13:117-252.

Vermoesen, A., H. Ramon, and O. Van Cleemput. 1991. Composition of the soil gas phase. Permanent gases and hydrocarbons. Pedology 41:119-132.

Wang, Z., R.D. DeLaune, C.W. Lindau, and W.H. Patrick, Jr. 1992. Methane production from anaerobic soil amended with rice straw and nitrogen fertilizers. Fert. Res. 33:115-121.

Wells, B.R., and F.T. Turner. 1984. Nitrogen use in flooded rice soils. In: Nitrogen in crop production. (Ed.) R.A. Hauk, Am. Soc. Agron. Madison, Wisconsin.

Whiting, G.J., J.P. Chanton, D.S. Bartlett, and J.D. Happel. 1991. Relationships between CH_4 emissions, biomass, and CO_2 exchange in a subtropical grassland. J. Geophy. Res. 96(D7):13,067-13,071.

Yagi, K., and K. Minami. 1990. Effect of organic matter application on methane emission from some Japanese paddy fields. Soil Sci. Plant Nutr. 36(4):599-610.

Yoshida, T.M., and M. Suzuki. 1975. Formation and degradation of ethylene in submerged rice soils. Soil Sci. Plant Nutr. 21:129-135.

CHAPTER 3

EFFECTS OF SOIL REDUCTION INTENSITY ON ROOT AERENCHYMA FORMATION AND METHANE AND OXYGEN EXCHANGE IN RICE

INTRODUCTION

The submergence or flooding of an aerobic soil results in successive reduction reactions that are dependent on the intensity of the reduction (Takai and Kamura, 1966). The predominant chemical changes initiated by flooding a soil include the disappearance of O_2, anaerobic decomposition of organic matter, transformations of N, reductions of Fe^{3+}, Mn^{4+} and $S0_4^{2-}$, and the accumulation of CO_2 or its reduction to CH_4 gas (Ponnamperuma, 1972). A flooded soil normally experiences redox potential (Eh) changes ranging from well oxidized to strongly reduced. Redox potential ranges from +400 to +600mV in well-aerated soils, and from -300 to +100mV in most reduced or anaerobic soils. Between this range, moderately reduced soil conditions exist with Eh's from 100 to 400 mV.

Methane gas is one of the greenhouse gases that is reported to exert significant effects on the global heat balance, thus causing a possible elevation of global surface temperature (Bouwman, 1991). Whereas Masscheleyn *et al.*(1993) observed that a soil Eh value of -150mV was critical for CH_4 production, Glinski and Stepniewski (1974) reported CH_4 formation during anoxic incubation and in non-flooded soils at an Eh as high as 220mV.

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The major sources of CH_4 production under submerged conditions are natural wetlands (Sebacher *et al.*,1985) and cultivated rice paddy fields (Cicerone and Oremland, 1988). Wetland-aquatic plants serve as direct conduits between reducing and oxidizing environments (Chanton *et al.*, 1992). Rice paddy fields account for about 20% of global CH_4 flux (Bolle *et al.*, 1986; Schutz *et al.*, 1989), and are considered to be an important source of atmospheric CH_4 because of increasing global harvest area of this crop (Food and Agricultural Organization of the United Nations, FAO, 1985). Holzapfel-Pschorn *et al.* (1986) investigated the effects of rice vegetation on CH_4 emission from submerged paddy soils and reported that whereas emission of CH_4 from unvegetated fields was insignificant, over 90% of the CH_4 emitted from rice vegetated fields was plant-mediated. Similar results were reported by Cicerone and Shetter (1983), who investigated the phenomenon of CH_4 transport through the aerenchyma of the rice plant.

In addition to the chemical changes induced by flooding in paddy soils, flooding also causes a number of responses in plant roots of which aerenchyma formation is one of the most obviously adaptive (Justin and Armstrong, 1987). The term *aerenchyma* refers to any tissue containing air-filled intercellular spaces or lacunae. A well-developed aerenchyma system in plants would ensure an efficient exchange of gases between the atmosphere and the soil environment. Such a mechanism would therefore promote O_2 transport from the atmosphere into the root rhizosphere to serve the dual purpose of maintaining aerobic metabolism of roots and microbes and restricting movement of potentially toxic substances such as Fe^{2+}, Mn^{2+} and H_2S into plant roots by oxidation (Armstrong and Armstrong, 1988). Most importantly, easy access of the rhizosphere to O_2 from the atmosphere via a well-developed aerenchyma system would enhance the oxidation of CH_4 gas in the rhizosphere and hence mitigate its flux into the atmosphere (Hanson, 1980). Several reports, including those of Armstrong (1971), Smirnoff and Crawford (1985), and

Pezeshki *et al.* (1991) agree that the exposure of cultivated and marshland species to flooded conditions could increase the amount of aerenchyma (air-space) in roots. On the contrary, John *et al.* (1974) concluded that aerenchyma formation in the rice is part of ordinary root growth and is not increased by poorly aerated surroundings.

Although the process of methanogenesis has been reported to occur under reducing conditions (Schutz *et al.*, 1989), there is no well-defined and specific link between the intensity of soil anaerobiosis (soil reduction status) and CH_4 production and emission. Neither is there any information in the literature linking soil reduction status to the degree of air-space formation and rhizosphere oxidation. It was hypothesized that increasing soil reduction intensity could induce changes in the plant, and also increase net emissions of CH_4 by enhancing methanogenesis and by modifying the diffusive pathway of gas transport. The objectives of this study were therefore to (1) evaluate the effect of increasing soil redox intensity on CH_4 production, (2) determine the extent to which specific levels of soil Eh could influence root air-space formation in rice (cv. *Rico*), and (3) determine if modifications in root air-space formation, in the rice plant response to soil redox intensity, influence gas transport.

MATERIALS AND METHODS

Incubation of soil suspension and soil redox equilibration

A Louisiana paddy soil (Crowley silt loam, Typic Albaqualf) was used in the present study. The plow layer (150mm depth) of the soil was collected from the Rice Research Station at Crowley, Louisiana, USA. It was air dried, ground, sieved through a 1mm mesh screen and then thoroughly mixed. The properties of the soil were described in the previous chapter (Chapter 2). Soil suspensions were prepared by mixing 400 g of the dry soil with 1400 mL deionized water. The soil was amended with finely ground rice straw at 13 ton ha^{-1} (to provide an energy source

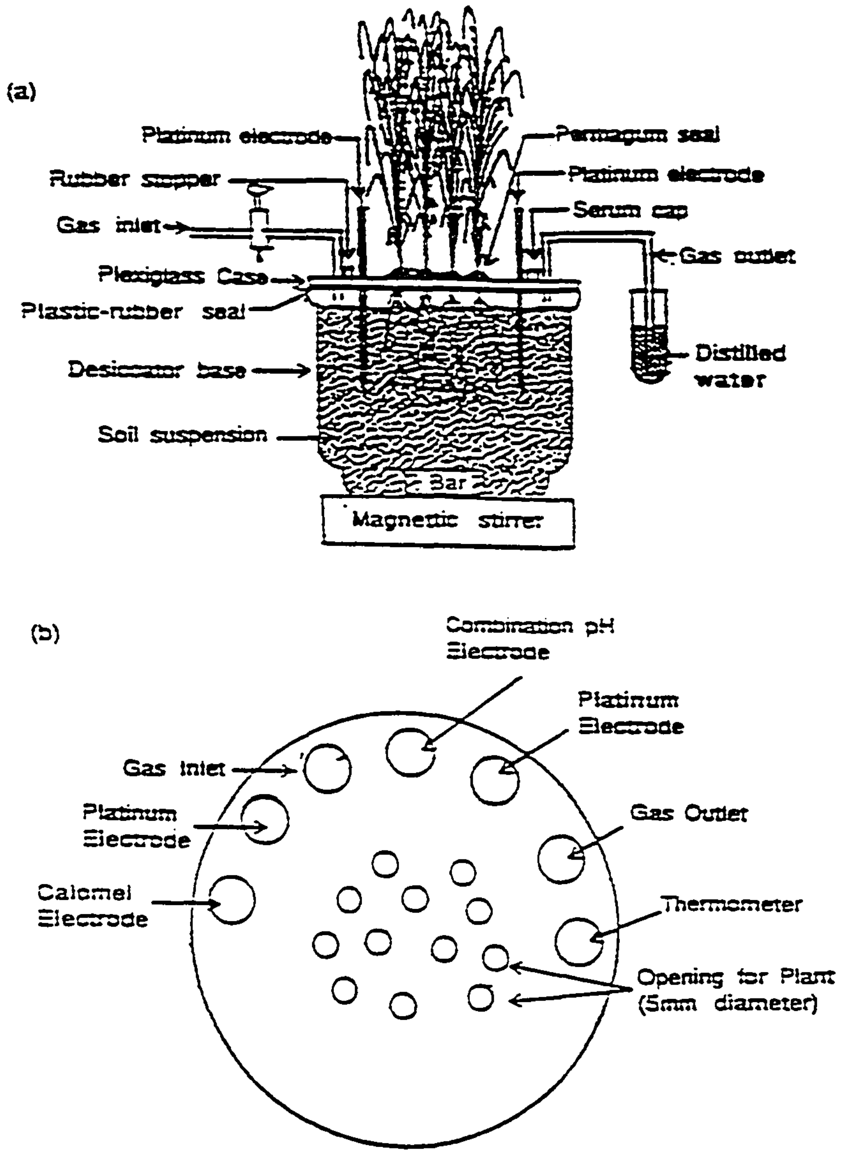

Figure 3.1: Diagrams of (a) growth set-up after transplanting seedlings, and (b) Plexiglass plate designed to support plants in the controlled redox system.

for microbial activity). The suspensions were equilibrated at 25±1 °C under controlled redox potential levels of +200, -200 and -300 mV in modified microcosms as described by Patrick and DeLaune (1977), Reddy *et al.* (1976) and outlined in Fig. 2.1. Each microcosm consisted of one 2-L pyrex glass desiccator flask (160mm id x 160mm high), a plexiglas plate and a redox control system. The plexiglas plate was designed for supporting transplanted plants, platinum and calomel electrodes, pH electrodes and a thermometer. The plate and desiccator were coated with aluminum spray to protect the soil suspension from light. In the redox control system, the soil Eh can be maintained at a selected level. The platinum electrodes that were permanently inserted through a rubber stopper of the plate were connected to a millivolt meter to give continuous measurements of the soil Eh. The recorder output of the millivoltmeter was connected to a meter relay that activated an air pump. Whenever the Eh dropped below the desired level, the air pump was automatically activated to pump air into the suspension to prevent the Eh from falling below the set value. During the equilibrations, argon gas was bubbled continuously through the soil suspension to remove O_2 and to promote anaerobic conditions in the suspensions. A magnetic stirrer was placed at the bottom of each flask to keep the soil in suspension. The use of this system provided a desired Eh within ± 20mV.

Plant material

Seeds of the rice cultivar *Rico* (medium-grain type) were soaked in 0.1% $HgCl_2$ for 60 s, rinsed with tap water and allowed to germinate and sprout in petri-dishes. They were then transferred to a nursery of aluminum trays containing air-dried sand and half-strength nutrient solution. The full strength nutrient solution contained 40 mg N L^{-1} as NH_4N0_3, 10mg P L^{-1} as $NaH_2PO_4.H_2O$, 40mg K L^{-1} as K_2SO_4, 40 mg Ca L^{-1} as $CaCl_2.2H_2O$, 40 mg Mg L^{-1} as $MgSO_4.7H_2O$ and traces of Mn, B, Zn, Cu and Fe as described by Yoshida *et al.* (1976). Twenty days after seed germination, 10 healthy seedlings were transplanted into each of the microcosms containing the incubated soil suspensions. Roots of the seedlings, that were trimmed to 5 cm, were threaded through the openings in the plexiglas plate into the soil suspensions. Plants were grown for a total of 50 days. An unvegetated pot, maintained at -300 mV, served as a control. The first CH_4 measurements were taken after 28 d. On the 50th day, coinciding with the panicle initiation stage of *Rico,* the following measurements were made: methane production and emission, root aerenchyma (air-space) development (POR), radial oxygen loss from roots (ROL), root length and shoot and root dry weights.

The incubation and culturing processes were repeated under the same laboratory conditions to allow for statistical analysis of data, using the randomized complete block design (RCBD), where the time factor constituted a block. Statistical analyses were performed using the PROC GLM and PROC CORR procedures in SAS (Statistical Analysis System, 1989).

Measurements

Methane gas production and emission

Prior to the determination of CH_4 fluxes from plants, the amount of CH_4 produced in soil suspensions was measured. For each soil redox potential treatment, a series of 8-mL soil suspension aliquots were removed from the flasks by means of a plastic syringe and needle and quickly introduced into evacuated 13-mL Vacutainers (Becton Dickinson, Lincoln Park, NJ) that were sealed with rubber stoppers. After injection of the soil suspension, the headspace of the Vacutainers was immediately flushed with helium gas at atmospheric pressure. The amount of CH_4 produced from quadruplicate soil suspension aliquots into the headspace was measured after every one hour for 8 h. Rates of CH_4 production were determined by regression analysis of CH_4 accumulation as a function of time. Prior to analyzing for CH_4, the Vacutainers were rigorously shaken on a vortex (Vortex-Gene 2) to transfer CH_4 from the soil suspension into the gas phase. After gas sampling, the Vacutainers were

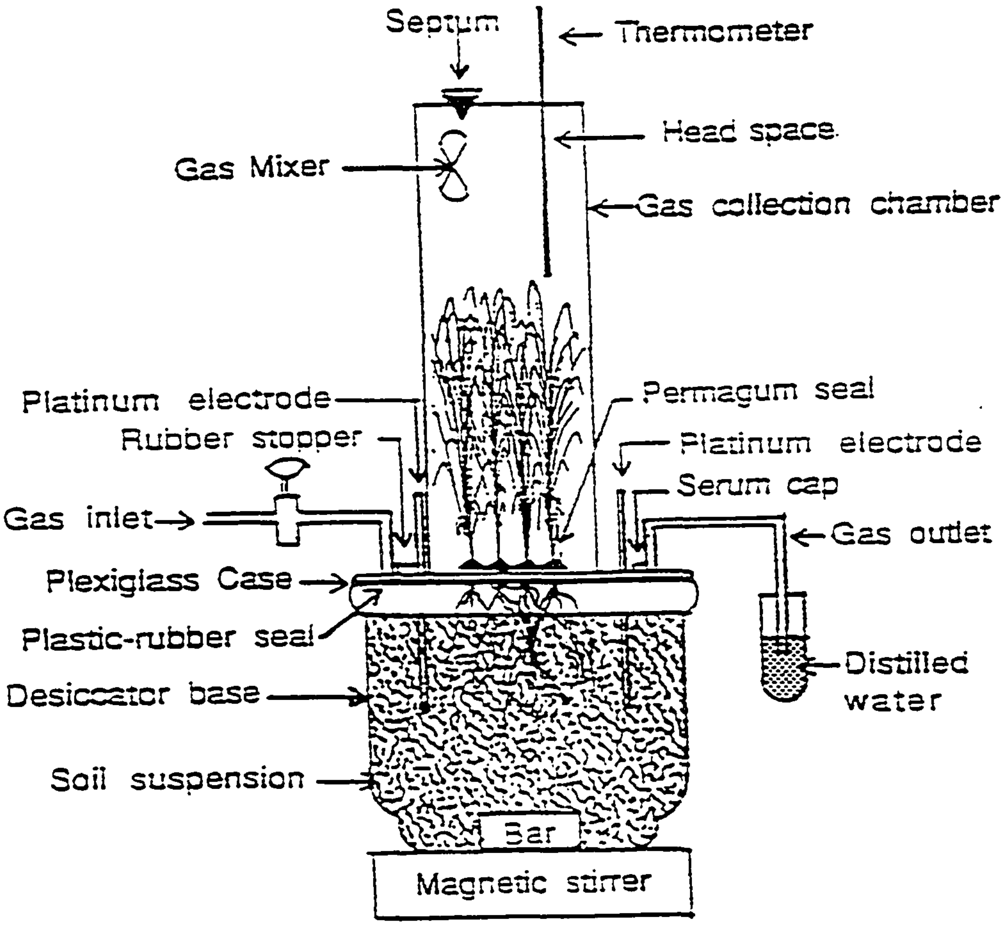

Figure 3.2: Diagram of gas-collection chamber enclosing rice plants for sampling CH_4 gas.

dried at 110 °C for 3 d and the amounts of dry soil and water in each were determined.

A modified closed chamber method, as described by Minami and Yagi (1988), was used for the measurement of CH_4 flux from rice plants. The chambers and sampling system are illustrated in Fig. 3.2. The chambers were constructed from plexiglas cylinders having 10 cm internal diameter with an effective height of 44 cm, as described in Chapter 2. Each gas collection chamber had a small fan fixed on the upper wall for homogenizing the air prior to sampling. A rubber septum, and thermometer were installed in the top of each chamber. Methane flux was determined by measuring the temporal increase of CH_4 concentration of air within the chamber. The chamber was mounted on top of the plexiglass plate enclosing all the 10 rice plants after flushing with argon gas. Air sampling was performed immediately after mounting the chamber and at one hour intervals for 8 h. Air temperatures inside and outside the sealed chamber were recorded and found to have less than 2 °C difference. All gas flows into the desiccator flasks were stopped during gas sampling procedures.

The CH_4 concentrations of the samples collected from both the Vacutainers and collecting chambers were determined using a Perkin-Elmer 900 gas chromatograph equipped with a flame ionization detector (Lindau *et al.*, 1991). Methane standards containing 10.5 and 110 μL CH_4 L^{-1} air were employed to construct a standard curve and the amount of CH_4 in the gas samples was determined. The production and flux rates of CH_4 from soil suspensions and rice plants respectively were the determined. Methane production rate was expressed in ng CH_4 pot^{-1} d^{-1} while CH_4 emission rate was in either ng CH_4 pot^{-1} d^{-1} or nmol CH_4 $plant^{-1}$ d^{-1}.

Radial oxygen loss and root porosity

The rate of O_2 released through rice roots was estimated colorimetrically with Ti^{3+}-citrate solution at 50 d as described in detail in Chapter 1. Measurements of the absorbance of the partly oxidized Ti^{3+}-citrate solution were made at 750 nm on a Perkin-Elmer 3 UV/VIS spectrophotometer. Released O_2 was determined by extrapolation of the measured absorbance to a standard curve previously obtained from a dilution series of the Ti^{3+}-citrate solution being used.

After ROL measurements, roots were rinsed with deionized water. Root porosity was measured by a pycnometer method (Jensen *et al.*, 1969) as descibed in detail in Chapter 1. At each sampling time, five plants were randomly harvested from each pot.

RESULTS AND DISCUSSION

Methane production was enhanced with decrease in soil Eh (Table 3.1). At -200mV, CH_4 production was comparatively low. However, a decrease of Eh from -200 mV to -300mV increased CH_4 production 10-fold in the vegetated treatment and about 9 times in the unvegetated pot. At the higher Eh of +200 mV, CH_4 production was only 0.7% of that from the -300 mV treatment. The fact that CH_4 was detected at this hypoxic condition(+200mV) is of interest. Schutz *et al.* (1989) reported that methanogenic bacteria can function only under completely reduced soil conditions, and according to Cappernberg (1975), maximum populations of CH_4 producing bacteria occur between -250 and -300 mV. It appears some amount of CH_4 is initially produced at this hypoxic stage because of the presence of acetic acid (synthesised by both facultative and obligate anaerobes, Tsutsuki,1984), a substrate for CH_4 production. This is consistent with the findings of Smith *et al.* (1978) who reported that both ethylene and CH_4 are consistently formed at the onset of the formation of Fe^{2+} and before major CH_4 formation. Nevertheless, results of this

Table 3.1: Rates of methanogenesis[1], CH_4 emission[1], and percentage of CH_4 emitted in laboratory cultures of rice after 50 days of soil incubation.

Soil redox potential	CH_4 production rate	CH_4 emission rate	CH_4 emitted
mV	ng pot^{-1} d^{-1}		%
-300	3232±43[1]	1438±35	44
-200	326±19	84±12	26
+200	23±05	6±0.2	24
-300 (unvegetated)	3106±36	nd[2]	nd

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[1] Measurements were conducted on three (for methanogenesis) and two (for CH_4) emission replicates. Values are means ± standard deviation.

[2] Data was not collected.

Table 3.2: Effect of soil redox potential (Eh) on root and shoot growth of rice after 50 days soil incubation[1].

Eh	Shoot dry weight	Root dry weight	Shoot:Root ratio	Mean root length
mV	g $plant^{-1}$			cm
-300	0.53±0.03	0.15±0.02	3.5a[2]	10.8±1.3
-200	1.30±0.13	0.75±0.04	1.8b	21.6±1.4
+200	1.60±0.13	0.80±0.03	2.0b	23.1±1.2

[1] Measurements were determined on five replicates and values are means ±standard deviation.

[2] Using the Duncan's Multiple Range test, values within a column followed by the same letter are not significantly different, ($P<0.05$).

study showed that methanogenesis is stimulated only at soil Eh levels at or below -200mV. This indicates that more CH_4 is produced with reduction in soil Eh as long as organic substrates are available. Rice paddy soils, characterized by oxygen depletion, high moisture and relatively high organic substrate levels offer an ideal environment for the proliferation of methanogens. The soil Eh may therefore be considered one of the most essential factors influencing the rate of CH_4 production in paddy fields. There was a 4% decrease in CH_4 production in the unvegetated pots. The disparity in production levels between the vegetated and unvegetated treatments may be ascribed to the additional contributions made by root exudates and root autolysis products to the existing organic substrates in the soil medium. These extra products are reported to contain sugars, amino acids and organic acids (Hale and Moore, 1979; Trolldenier, 1981) which constitute readily available carbon and energy sources for the soil microbial community, including methanogenic bacteria (Kimura, *et al.*, 1991).

At 28 d, the rice cultivar *Rico* was at its tillering stage of growth. The total amounts of CH_4 emitted at this stage were 4.06, 0.31 and 0.02 nmol CH_4 plant^{-1} d^{-1} at the Eh levels of -300, -200 and +200 mV respectively (Fig 3.3). These results followed a trend similar to that of CH_4 production rate and may suggest a high positive correlation between CH_4 production and its emission: the higher the amount of CH_4 produced, the higher the amount that is transported to the atmosphere through rice plants. An enhancement of CH_4 emission rate was recorded at 50 d, especially with respect to the -300 mV treatment: emission rate increased from 4.06 to 9.0 mmol CH_4 plant^{-1} d^{-1}. Cicerone and Shetter (1983) and Seiler *et al.* (1984) have demonstrated that more than 95% CH_4 released from rice paddies is due to transport through the rice plant. Methane produced in the anaerobic soil enters the aerenchyma of the root system of plants (Drew *et al.*, 1979; Justin and Armstrong, 1987) and the influx of CH_4 might be facilitated by a diffusion gradient between

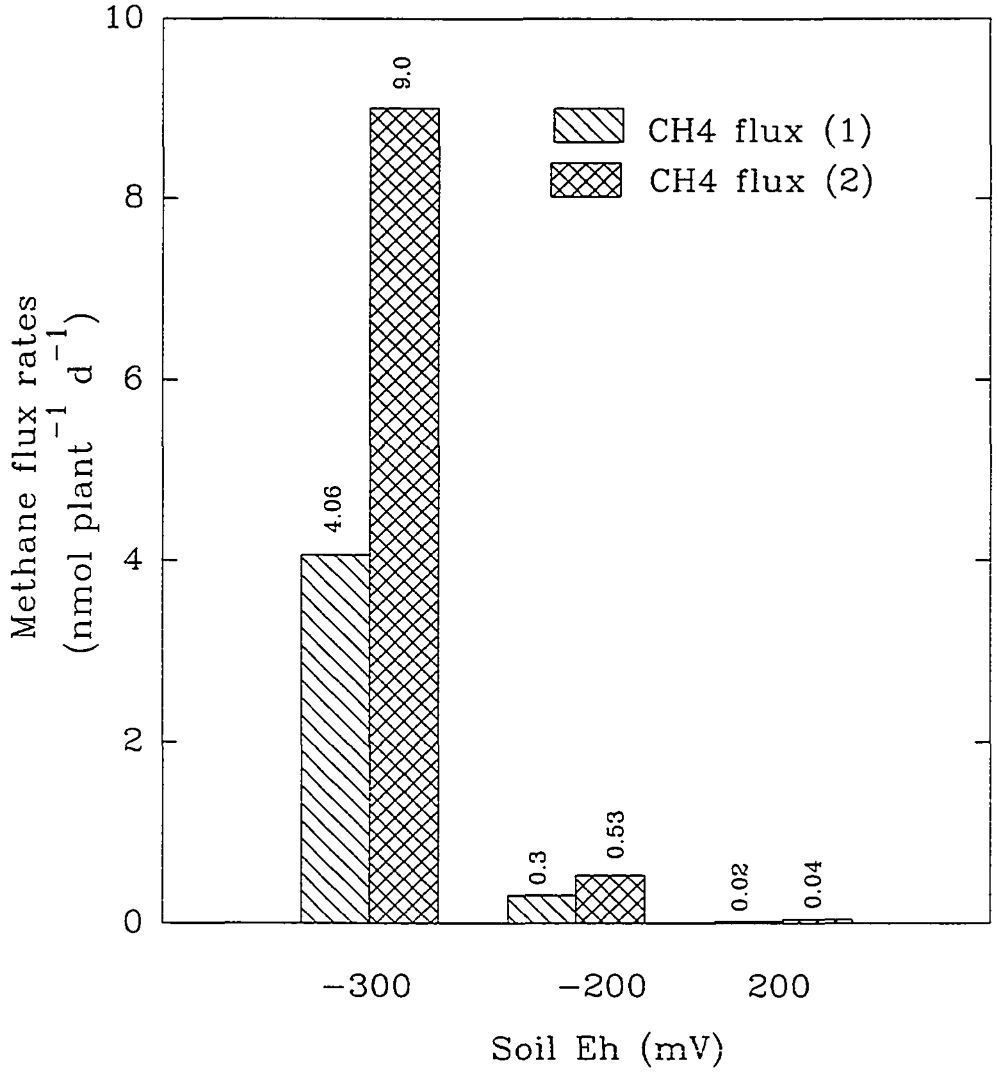

Figure 3.3: Rates of CH_4 emission from rice plants as a function of soil redox potential (Eh) at (1) 28 and (2) 50 days of soil incubation.

soil/sediment and the atmosphere, because of the capacity of the internal gas phase to take up CH_4 is infinite as long as the gas phase is connected to the atmosphere (Schutz *et al.*, 1991). In this study, the rate of methane flux that could result from ebullition and diffusion (Holzapfel-Pschorn *et al.*, 1986) was not determined. It was assumed that all CH_4 emitted from the vegetated microcosms was plant-mediated since there was no other escape route for CH_4 from the soil suspensions into the gas collecting chambers.

Figure 3.4 depicts root air-space formation as a function of soil redox potential. In general, the intensity of reduction influenced root air space formation. At -300 mV, plant roots had 35±6% root air space compared to 30.7±3% and 26.8±2% at -200 mV and +200 mV, respectively. Van der Heide *et al.* (1963) reported that some degree of O_2 stress in meristematic regions of roots may always be necessary to trigger gas space development. In this study, it appears that not only was gas-space formation triggered by anaerobiosis but was actually enhanced by the intensity of anaerobiosis. This is contrary to the report of Luxmoore and Stolzy (1969) who found no effect of soil O_2 concentration on root porosity in *Oryza sativa* and *Zea mays*. However, by comparing the relative amounts of CH_4 production, CH_4 emission and root porosity (POR) at the respective soil Eh levels, it appears the intensity of anaerobiosis influences methanogenesis more than root air-space formation, CH_4 flux being dependent on the two. This suggests that rate of methanogenesis may be the more important factor controlling CH_4 fluxes under the experimental conditions. Seiler *et al.* (1984) reported that the flux rate of CH_4 from paddy soils into the atmosphere may be limited either by transport into the root system or transport through the aerenchyma system and/or by CH_4 production rate within the paddy soil. The results of this study seem to suggest that the latter factor may be the most limiting factor that affects CH_4 flux from the soil into the atmosphere.

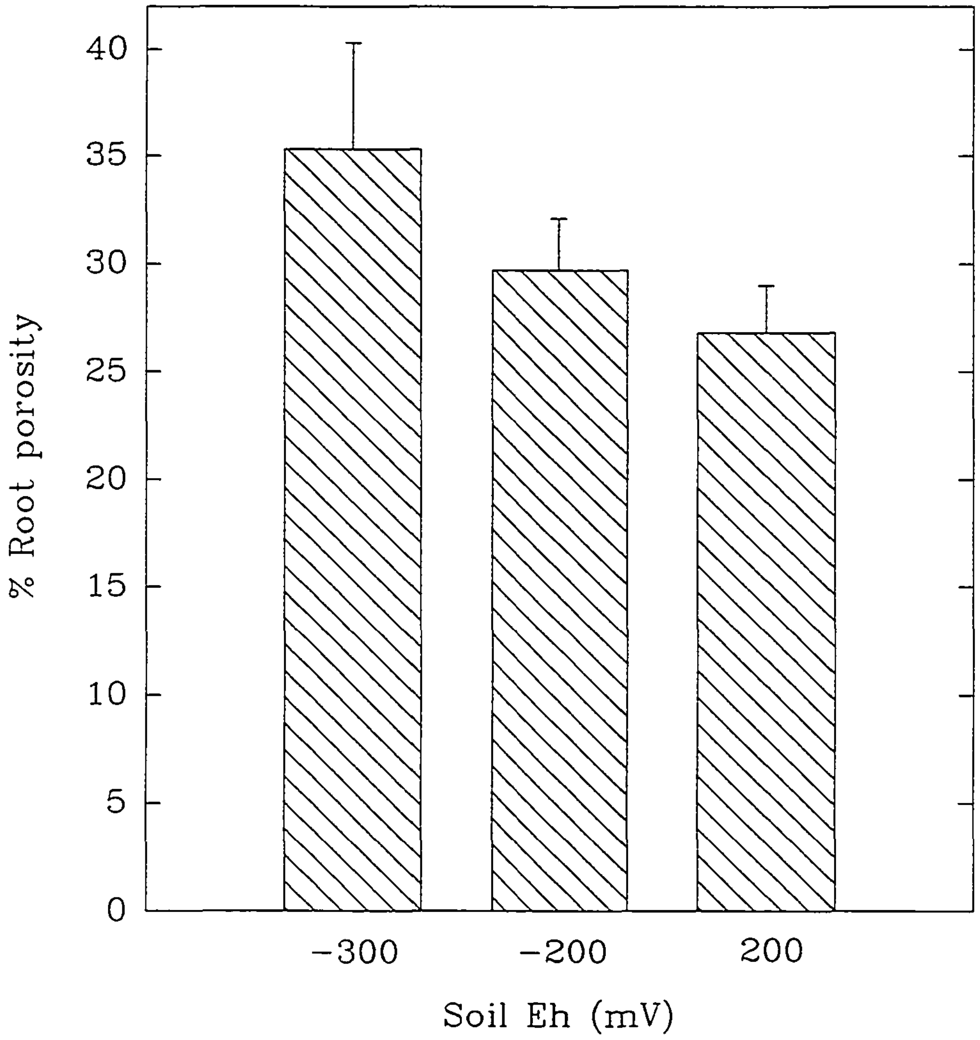

Figure 3.4: Root porosity in rice porosity as a function of soil redox potential.

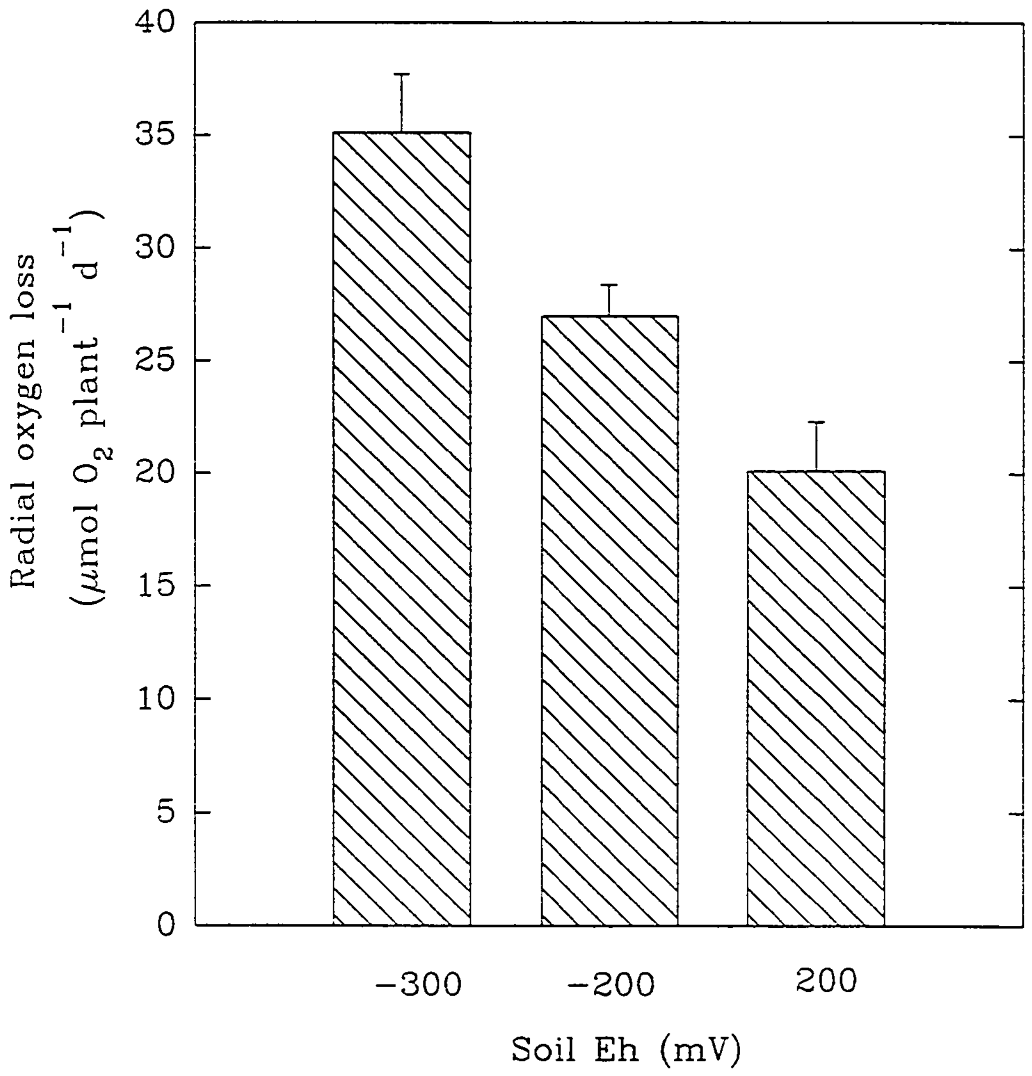

Figure 3.5: Radial oxygen loss (ROL), from rice roots as a function of soil redox potential (Eh).

The rate of ROL as a function of soil Eh is shown in Fig 3.5. Radial oxygen loss showed a similar trend as POR, with a high correlation ($r=0.96$) between these two parameters, and confirming the earlier report on *Rico*, (Chapter 1). Plants grown in the more reduced soils exhibited higher O_2 loss from roots. A plausible explanation for this observation is that plants grown under more reducing conditions such as -300 mV developed more extensive gas space with a reciprocal decrease in O_2 demand by the root cells (Williams and Barber, 1961) and with reduction in diffusional resistance. According to Luxmoore *et al.* (1970), and Armstrong (1979), the effectiveness of gas transport is dependent on (1) the physical resistance to diffusion (which is directly proportional to root length and is inversely proportional to fractional root porosity), and (2) O_2 demand along the diffusion path (which is a function of respiratory uptake and radial O_2 leakage from the root to the soil). As shown in Table 3.2, plants grown under the severe reducing condition of -300 mV were adversely affected: compared with the other treatments, they had lower shoot and root dry weights and mean root length. A lower root weight implies less respiring tissue as reflected in the higher air-space development; and shorter roots also implies less tortuosity and lesser O_2 demand in the highly reduced environment. It is therefore suggested that the higher rate of gas exchange in plants cultured under -300mV resulted from both physiological and morphological changes within the plant.

Under reduced soil conditions, the net amount of CH_4 emitted is the result of two opposing processes-CH_4 production and its oxidation. The rates of CH_4 production and emission and the percentage of emitted CH_4 at their corresponding Eh levels are shown in Table 3.1. The difference in CH_4 production and emission rates may be attributed to the oxidation of CH_4 in the rhizosphere with the O_2 originating from leakage or conductance from the roots. Holzapfel-Pschorn *et al.* (1986) and Sass *et al.* (1990) reported that between 58% to 80% of CH_4 produced was oxidized by methanotrophic bacteria and not emitted to the atmosphere. Compared to those reported results, the rate of CH_4 reoxidation appeared to be lower in this study. This probably occurred because of lower rhizosphere:soil ratio (v/v) of the microcosms, and also the diversion of some of the O_2 that leaked into the root rhizosphere towards the reoxidation of Fe^{2+}, Mn^{2+}, H_2S and other reductants formed under reducing conditions. Gambrell and Patrick (1978) reported that about 50% of O_2 consumption in soils and sediments was due to reoxidation of chemical reductants. It is suggested that part of the available O_2 in the rhizosphere was used for the oxidation of the chemical reductants in greater preference to CH_4 oxidation probably because of differences in kinetics of reductant-reoxidation and also possibly because of a shorter residence time of CH_4 in the rhizosphere. There is the need to determine the kinetics and the residence time of CH_4 in the rhizosphere.

CONCLUSIONS

The results of this study demonstrate that soil Eh exhibits complex interactions with the physiology of the rice plant and bacterial production of CH_4 and its net emission. Lower levels of soil Eh resulted in reduction of root length and weight but enhancement of root porosity, culminating in gas exchange promotion. Methane production and emission rates increased sharply with decreasing soil redox status. Apparently, CH_4 oxidation played some role in this study since only a fraction of the amount produced was evolved. Methane emission from rice paddies is therefore a function of the rate of methanogenesis, aerenchyma formation and rate of methane oxidation. However, since laboratory studies may not necessarily reflect actual conditions in paddy fields, and since the entire growth period of rice was not covered, results of this study cannot be extrapolated to cover global scale of CH_4 emission. Nevertheless, the study provides a theoretical basis for understanding the influence of soil Eh on rice growth, CH_4 production and gas exchange. Apart from soil redox status, other soil factors (e.g. soil pH, and concentration of soil reductants) and management practices (e.g. organic matter application, and rice cultivar) may also influence those responses in paddy rice. It is therefore pertinent to investigate the interactions of these factors and practices in future studies for determining measures of mitigating CH_4 emission.

REFERENCES TO CHAPTER 3

Armstong, J. 1979. Aeration in higher plants. Adv. Bot. Res. 7:697-708.

Armstong, J. 1971. Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration and waterlogging. Physiol. Plant. 25:192-198.

Armstong, J., and W. Armstrong. 1988. *Phragmites australis.* A preliminary study of soil oxidizing sited and internal gas transport pathways. New Phytol. 108:373-382.

Bolle, H.J., W. Seiler, and B.Bollin. 1986. Other greenhouse gases and aerosols. In: The greenhouse effect, climate change and ecosystems, Ch. 4 (SCOPE 29) (J. Willey & Sons, N.Y., 1986), pp. 157-198.

Bouwman, A.F. 1991. Agronomic aspects of wetland rice cultivation and associated methane emissions. Biochem. 15:65-88.

Cappenberg, T.E. 1975. Relationships between sulfate-reducing and methane producing bacteria, Plant Soil. 43:125-139.

Chanton, J.P., G.J. Whiting, W.J. Showers, and P.M. Crill.1992. Methane flux from Peltandra virginica: Stable isotope tracing and chamber effects. Global Biogeochem. Cycles. 6(1):15-31.

Cicerone, R.J., and R.S. Oremland. 1988. Biogeochemical aspects of atmospheric methane. Global Biogeochem. Cycles 2:299-327.

Cicerone, R.J., J.D. Shetter. 1983. Seasonal variation of methane flux from California rice paddy. J. Geophys. Res. 88(c):11022-11034.

Drew, M.C., M.B. Jackson, and S. Giffard. 1979. Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. Planta 147:83-88.

Food and Agriculture Organization of the United Nations. 1985. Fertilizer Yearbook, Vol. 35. FAO Statistical series No. 71, FAO, Rome, Italy.

Gambrell, R.P., and W.H. Patrick Jr. 1978. Chemical and microbiological properties of anaerobic soils and sediments. p. 375-423. In D.D. Hook and R.M.M. Crawford (ed.) Plant Life in Anaerobic Environments. Ann Arbor Sci. Publishers Inc. Ann Arbor. Mich. USA.

Glinski, J., and W. Stepniewski. 1983. Soil aeration and its role for plants. CRC Press. Inc. Boca Raton. Florida pp. 173-188.

Hale, M.G. and L.D. Moore. 1979. Factors affecting root exudation II:1970-1978. Adv. Agron. 31:93-124.

Hanson, R.S. 1980. Ecology and diversity of methylotrophic organisms. Adv. Appl. Microbiol. 26:13-40.

Holzapfel-Pschorn, A., R. Conrad, and W. Seiler. 1986. Effects of vegetation on the mission of methane from submerged paddy soil. Plant Soil 92:223-23.

Jensen, C.R., R.J. Luxmoore, S.D. Van Gundy, and L.H. Stolzy. 1969. Root air-space measurements by a pycnometer method. Agron. J. 61:474-475.

John, C.D., V. Lemipinuntana, and H. Greenway. 1974. Adaptation of rice to anaerobiosis. Aust. J. Plant Physiol. 1:513-520.

Justin, S.H.F.W., and W. Armstrong. 1987. The anatomical characteristics of roots and plant response to soil flooding. New Phytolol. 106:465-495.

Kimura, M.D., H. Murakami, and H. Wada. 1991. CO_2, H_2, and CH_4 production in rice rhizosphere. Soil Sci. Plant Nutri. 37:55-60.

Lindau, C.W., P.K. Bollich, R.D. DeLaune, W.H. Patrick, Jr., and V.J. Law. 1991. Effect of urea fertilizer and environmental factors on CH_4 emissions from Louisiana, USA rice field. Plant Soil 136:195-203.

Luxmoore, R.J., and L.H. Stolzy. 1969. Root porosity and growth responses of rice and maize to oxygen supply. Agron. J. 61:202-204.

Luxmoore, R.J., L.H. Stolzy, and J. Letey. 1970. Oxygen diffusion in the soil plant system. I.A. Model. Agron. J. 62:317-325.

Masscheleyn, P.H., R.D. DeLaune, and W.H. Patrick, Jr. Methane and Nitrous Oxide Emissions from a flooded Rice Soil: Effect of Soil Oxidation-Reduction status. Chemosphere 26:251-160.

Minami, K. and K. Yagi. 1988. Method of measuring methane flux from rice paddies. (In Japanese.) Jpn. J. Soil Sci. Plant Nut. 59:458-463.

Patrick, W.H., Jr., and R.D. DeLaune. 1977. Chemical and biological redox systems affecting nutrient availability in the coastal wetlands. Geosci. and Man. 18:131-137.

Pezeshki, S.R., S.W. Mathews, and R.D. DeLaune. 1991. Root cortex structure and metabolic responses of Spartina patens to soil redox conditions. Environ. Exp. Bot. 31(1):91-97.

Ponnamperuma, F.N. 1972. The chemistry of submerged soils. Adv. Agron. 24:29-96.

Reddy, C.N., A. Jugsujinda, and W.H. Patrick, Jr. 1976. System for growing plants under controlled redox potential-pH conditions Agron. J. 68:987-989.

Sass, R.L., F.M. Fisher, P.A. Harcombe, and F.T. Terner. 1990. Methane production and emission from a Texas rice field . Glob. Biogeochem. Cycles. 4:47-68.

Schutz, H., P. Schroder, and H. Rennenberg. 1991. Role of plants in regulating the methane flux to the atmosphere. pp. 29-63. In: Trace Gas Emissions by Plants. Academic Press Inc. San Diego.

Schutz, H., Seiler, W., and R. Conrad. 1989. Processes involved in formation and emission of methane in rice paddies. Biogeochem. 7:33-53.

Sebacher, D.I., R.C. Harris, and K.B. Barlett. 1985. Methane emissions to the atmosphere through aquatic plants. J. Environ. Qual. 14:40-46.

Seiler, W., A. Holzapfel-Pschorn, R. Conrad, and D. Scharffe. 1984. Methane emission from rice paddies. J. Atmos. Chem. 1:241-268.

Smirnoff, M., and R.M.M. Crawford. 1983. Variation in thestructure and response to flooding of root aerenchyma in some wetland plants. Ann. Bot. 51:237-249.

Smith, A.M., P.J. Milham, and W.L. Morrison. 1978. Soil ethylene production specifically triggered by ferous iron. p. 269-272. In: M.W.Loutit and J.A.R. Mile. (ed.) Microbial Ecol. Springer verlag, New York.

Statistical Analysis System (SAS). 1989. SAS/SAT User's Guide for personal computers. SAS Institute Inc., Cary, NC.

Takai, J. T., and T. Kamura. 1966. The mechanism of reduction in waterlogged paddy soil. Folia Microbiol. 11:304-313.

Trolldenier, G. 1981. Influence of plant metabolism on microbial activity in the rhizosphere. Proc. 16th Coll. Int. Potash Institute, Bern 1981 137-146.

Tsutsuki, K. 1984. Volatile products and low molecular weight phenolic products of the anaerobic decomposition of organic matter. p. 329-343. In: Organic matter and Rice. IRRI 1984. IRRI, Los Banos, Laguna, Philippines.

Van der Heide, H., B.M. Boer-Bolt, and M.H. Van Raalte. 1963. The effect of low oxygen content of the medium on the roots of barley seedlings, Acta Bot. Neerl. 12:231-238.

Williams, W.T., and D.A. Barber. 1961. The functional significance of aerenchyma in plants. Symp. Soc. Exp. Biol. 15:132-144.

Yoshida, S., D.A. Romo, J.H. Cock, and K.A. Gomez. 1976. Laboratory manual for physiological studies of rice. 3rd ed. IRRI. Los Banos, Philippines.

CHAPTER 4

METHANE EMISSIONS AND PLANT GROWTH OF WIREGRASS IN RESPONSE TO SOIL REDUCTION INTENSITY

INTRODUCTION

Natural wetlands constitute a major source of CH_4 production (Sebacher *et al.*, 1985), accounting for over 100 million tonnes of CH_4 annually (Blake and Rowland, 1988; Watson *et al.*, 1990). Vegetation in wetlands controls CH_4 production and flux by providing substrates for methanogenesis through input of litter and root exudates, and by serving as conduits for gas exchange (Holzapfel-Pschorn *et al.*, 1986; Schutz *et al.*, 1989). Methane emissions from wetlands are highly variable and are determined by complex interactions among the chemical, physical and biological properties of the local environment (Bartlett *et al.* 1985). Several environmental factors including soil oxidation-reduction potential (Eh) (Wang *et al.*, 1993; Kludze *et al.*, 1993) are known to influence CH_4 production and emissions.

In response to reducing conditions, most hydrophytes develop the aerenchyma system as an adaptation mechanism to allow or enhance continued growth and survival (Armstrong, 1979; Keeley, 1979). The primary function of these air-spaces appears to be the delivery of O_2 into roots; as such, they represent an extension of the atmosphere into soils or sediments. However, in the process of bringing O_2 down to their submerged roots, hydrophytes transport CH_4 from the sediments to the atmosphere (Sebacher *et al.*, 1985; Chanton *et al.* 1989; Kludze *et al.*, 1993).

The magnitude and significance of wetland plant species as a vehicle of CH_4 emission have limited documentation. There is the need to improve our understanding of the different types of ecosystems that control CH_4 dynamics. Studies conducted by Whiting *et al.* (1991), Sebacher *et al.* (1985), and Dacey and Klug (1979), on some wetland species indicated that the movement of CH_4 from anaerobic sediments through the shoots of these plants into the atmosphere could provide a significant pathway for the emission of CH_4, the net amount being dependent on CH_4 production, oxidation and the transport system. *Spartina patens* (wiregrass) is a perennial flood-tolerant grass that dominates some dune, swale and marsh habitats in coastal Louisiana. The species is distributed continuously along the US coast from Maine to southern Texas, and is also found in the West Indies, Cuba, and the Yucatan Peninsula. Although it is well acknowledged that methanogenesis occurs under strongly reducing conditions, the link between soil redox status and CH_4 emissions from *S. patens,* in conjuction with the plant's physiological responses, has little or no documentation. The objectives of this study were to: (1) evaluate the effect of soil reduction intensity on CH_4 production in *S. patens* culture. (2) determine the changes in root porosity in response to soil redox intensity and plant age (corresponding to sampling time), (3) determine if any increases in root porosity in response to soil redox intensity influences gas transport in *S patens.*

MATERIALS AND METHODS

Plant materials

Spartina patens plants were collected from brackish marshes of Louisiana Barataria Basin and were transferred to a greenhouse. Newly germinated propagules were separated from mature plants and nursed in pots filled with commercial potting soil (Jiffy mix plus, Jiffy Products of America, Chicago, IL). After a 3-week growth period, propagules of about the same height were selected and carefully washed of all soil particles and then transferred to half-strength nutrient solution as described by Yoshida *et al.* (1976). Plants were grown in the nutrient solution for 3 extra weeks under the following laboratory conditions: day and night temperatures, 25±2 °C, 24-h photoperiod with light intensity of 1000-1200 μmol m^{-2} s^{-1} at canopy level. The nutrient solution was changed once a week.

Soil incubation and soil redox equilibration

Three hundred grams of Mhoon soil, Mississippi alluvial silty-clay loam (Typic Fluvaquent) were weighed into 2-L glass desiccator flasks and thoroughly mixed with water (1:5 soil:water ratio) to form a soil suspension. The soil was originally air dried, ground and sieved through a 1 mm mesh screen. Selected properties of the soil included pH of 6.5 (1:1 soil/water ratio), bulk density of 1.5 g per cm^3, clay content of 300 g kg^{-1}, silt content of 620 g kg^{-1}, and organic matter content of 12 g kg^{-1}. The suspensions were equilibrated at 25 ± 2 °C under controlled redox potential levels of +200, -200 and -300 mV in modified microcosms developed by Reddy *et al.* (1976) and described in detail in Chapter 3; (also see Kludze *et al.* (1993)). Treatments (Eh levels) were assigned randomly to the desiccator flasks.

Each desiccator flask (microcosm) containing incubated soil had 10 plants. The plants (healthy propagules of wiregrass) were transplanted into the incubated soil suspensions by threading their roots through the openings in the plexiglas plate into the soil suspensions. The plants were grown for a total of 50 days. The entire experiment, consisting the incubation and culturing processes, was repeated under the same laboratory conditions to allow for statistical analysis of data, using the randomized complete block design, where the time factor constituted a block. All data were subjected to analysis of variance using the General Linear Models and Duncan's multiple range test procedures of the Statistical Analysis System (SAS Institute, 1989).

Measurements

Methane gas production, oxidation and emission

Prior to the determination of CH_4 fluxes from plants, the amount of CH_4 produced in the soil suspensions was measured, using the method described in detail in Chapter 3, (also see Kludze *et al.* (1993)). Rates of CH_4 production were determined by regression analysis of CH_4 accumulation as a function of time and expressed on the basis of total soil solution per desiccator flask. The closed-chamber method with sampling system as described in detail by Kludze *et al.*, (1993), was used to sample CH_4 emissions. Diurnal emissions were determined under light (1,000-1,200 μmol m^{-2} s^{-1}) at 20, 30, 40 and 50 d after transplanting. Methane emissions in the dark were measured simultaneously within the same time period to determine if emissions compared with day-time emissions. The chambers used for the dark measurements were constructed by coating the outer surface of the original chambers with aluminum foil. All gas flows into the desiccator flasks were stopped during gas sampling procedures.

Methane samples originating from both production and emissions were measured by using a Perkin-Elmer 900 gas chromatograph equipped with a flame ionization detector. Methane standards containing 10.5 and 110 μL CH_4 L^{-1} air were employed to construct a standard curve and the amounts of CH_4 in the gas samples were determined. Methane production and emission rates were expressed in ng CH_4 pot^{-1} d^{-1}. The difference between the two rates for each treatment was assumed to be the amount of CH_4 oxidized by plants. To confirm this, an independent study was conducted to quantitatively measure plant mediated oxidation of CH_4 under the same laboratory conditions. Methylfluoride (CH_3F), known to inhibit O_2 consumption by methanotrophs, was used. Soil suspension aliquots (120 mL) from incubated pots at -200 and -300 mV were dispensed into 150-mL evacuated flasks and kept in continuous suspension with magnetic stirrers. Thus, the set-up was a miniature of the controlled redox system. There were 12 flasks for each incubated pot; 6 of the flasks were injected with 0.15 mL (0.10 %) CH_3F, while the other 6 were without CH_3F. The inhibitor treatments were imposed 15 d after preconditioned *Spartina patens* plants that had been growing under Eh of -200 and -300 mV for 25 d were transferred into the flasks. Thus, plants were grown for a total of 40 d prior to data collection. The plants were placed through holes (1 per flask) in a plastic disc plate designed to fit the opening of the flasks. The plastic disc was securely glued onto the flask opening to prevent atmospheric air contamination. Triplicate controls, with or without plants, had no CH_3F treatment. Gas was collected an hour after CH_3F application in a 110 mL centrifuge tube (Nalge Company) placed over the mouth of each flask and analyzed for CH_4 as described earlier. Gas sampling was done after every 1 h for 8 h.

Radial oxygen loss (ROL) and root porosity (POR)

Radial oxygen loss (ROL) from roots was quantified colorimetrically at the end of the experiment by using Ti^{3+}-citrate solution as described in detail in Chapter 1. Root aerenchyma development was monitored throughout the experimental period at 10 d intervals for 50 d. The pycnometer technique (Jensen *et al.* (1969); Chapter 1)) was used to quantify fractional air-space formation in roots. Root and shoot dry weights, and root length were subsequently determined at the end of the experiment.

RESULTS AND DISCUSSION

Growth responses

All plant growth parameters studied (root elongation, shoot and root dry weights and shoot height) were adversely affected by the intensity of anaerobiosis (Table 4.1). For example, root dry weights decreased by 40% and shoot dry weights by 25% between the soil redox potential treatments of 200 mV and -300 mV. These decreases reflect more severe effects of redox intensity on root growth than on shoot growth. The inhibition of root growth (both in weight and length) observed in response to the intensity of anaerobiosis is in accord with previous findings. Pezeshki and DeLaune (1990) reported cessation of root growth at soil Eh around -100 mV while Pezeshki *et al.* (1991) also reported the formation of smaller root system in *S. patens* in response to anaerobiosis. Such a reduction in the root system would lead to

Table 4.1: Response of plant parameters to changes in soil redox intensity in *Spartina patens* at 40 days after transplanting. Values are means of 5 replicates per treatment.

Soil redox potential (mv)	Shoot dry weight	Root dry weight	Shoot:root ratio	Root length	Shoot height
	mg plant^{-1}			cm plant^{-1}	
-300	469.6b[1]	30.6c	15.3a	9.5b	33.9b
-200	485.0a	51.4b	9.4b	10.3b	39.0a
+200	487.0a	76.5a	6.4c	14.4a	43.8a

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[1] Using Duncan's Multiple Range test, values within a column followed by the same letter are not statistically different ($P<0.05$).

a reduction in sink size for photosynthate and cause a feed-back inhibition on photosynthesis and hence growth. Stolzy *et al.* (1961) noted that plant root growth was a function of soil oxygen content. Soil suspensions at the Eh levels used in our study were devoid of O_2, since O_2 completely disappears at redox potential values of about 350 mV and lower (DeLaune and Pezeshki, 1991), and since any O_2 conducted into the soil from the atmosphere via the roots would be readily scavenged by reduced chemical species.

Root porosity (POR) and radial oxygen loss (ROL)

Soil Eh significantly ($p<0.01$) affected root porosity in *S. patens* (Table 4.2). Root porosity increased with decrease in soil Eh and with increase in soil incubation period until at about 30 d, when further air-space formation appeared to level off and cease (Fig. 4.1). By day 30, POR ranged between 22% in the plants grown at +200 mV and 45% in plants grown at -300 mV treatments. The growth of wetland plants in anaerobic conditions requires development of an extensive internal aeration system allowing transport of atmospheric O_2 to the roots as a means of avoiding root anaerobiosis (Webb and Jackson, 1986). A number of studies of adapted wetland species have noted an increase in aerenchyma formation in response to flooding (Seliskar, 1988; Burdick and Mendelssohn, 1990). In our study, not only was gas-space formation triggered in response to flooding *per se* but was actually enhanced by the intensity of anaerobiosis. This observation is consistent with an earlier report on *Oryza sativa* (Chapter 2; Kludze *et al.* (1993). However, the fact that root root porosity in all treatments, ceased to increase by day 30, irrespective of the soil redox status, is of interest. The reason for the probable inhibition is unknown and needs further investigation. It is however suggested that although root porosity in *S. patens* is enhanced by the intensity of anaerobiosis, such an enhancement is limited to a specific period of time under reducing soil conditions as severe as those imposed in this experiment.

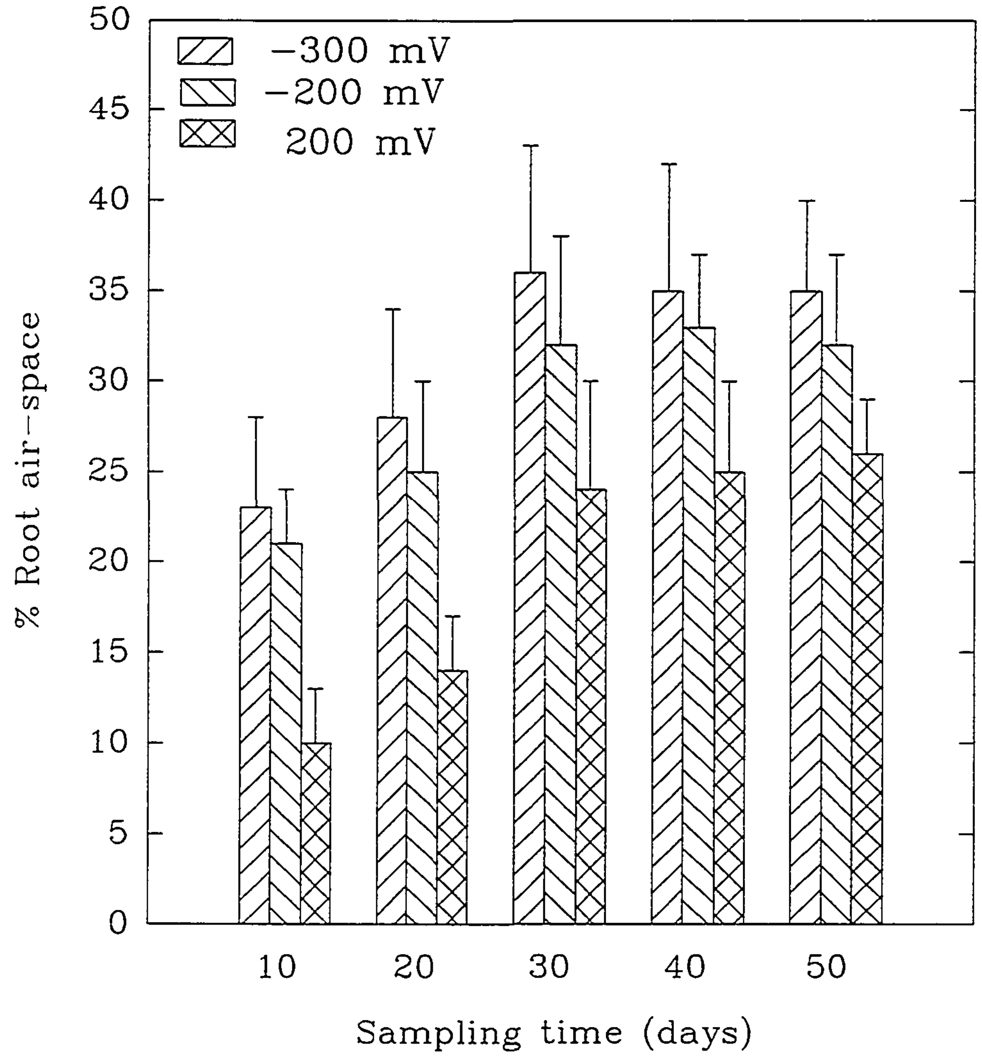

Figure 4.1: Root porostiy in *Spartina patens*, as a function of soil redox intensity (Eh) over the experimental period of 50 d. Bars represent ± standard deviation.

Table 4.2: Effect of soil redox intensity (Eh) and sampling time on root porosity (POR).

Factor		POR
Soil Eh (mV)		
	-300	33.10a[1]
	-200	30.30b
	200	24.3c
Sampling time (days)		
	10	19.8c
	20	23.7b
	30	32.4a
	40	33.7a
	50	32.8a
F-test		
	Soil Eh (T)	**
	Sampling time (S)	**
	(T) x (S)	NS[2]

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[1] Values followed by the same letter in a column under each factor are not significantly different ($p<0.05$), Duncan Multiple Range Test.

[2] NS=not significant; ** significant at $p<0.01$

There were differences in radial oxygen loss (ROL) between the reduced (+200 mV) treatment and the highly reduced treatments (-200 and -300 mV) as indicated in our ROL measurements (Fig. 4.2). No significant differences in O_2 release were however found between plants at -200 mV and -300 mV ($p < 0.05$) at 50 d of plant growth. The status of soil aeration influences root O_2 supply due to changes in gradient of O_2 concentration between the aerial parts and the rhizosphere (Gleason and Zieman, 1981; Yamasaki 1987). Low soil redox potentials result in low redox potentials of root cells (Schat, 1984). Lesser amounts of O_2 were released into the rhizosphere at 200 mV compared to the releases at the other treatments most likely because of the presence of more living root cells that require O_2 for aerobic respiration and/or because of the lesser air-space formed at that Eh. As suggested by Armstrong *et al.* (1991), there is always a competition for O_2 between root cells and the rhizosphere.

The hypothesis that increases in aerenchyma can provide sufficient O_2 for aerobic root metabolism under hypoxic/anoxic conditions has been suggested by Luxmoore *et al.* (1972) and confirmed in corn by Drew *et al.* (1985). However, despite the existence of an extensive aerenchyma system for O_2 supply, this system may be overwhelmed under extreme anaerobiosis when decrease in Eh is rapid and the reducing conditions are intense (Pezeshki and DeLaune 1990). Burdick and Mendelssohn (1990) reported that although maximum amount of aerenchyma (about 50%) had been formed in flooded roots of *S. patens* by day 29, alcohol dehydrogenase (ADH) activity on day 63 was still greater in flooded than in drained roots. They therefore suggested that the maximum amount of aerenchyma in *S. patens* was not sufficient to provide the root system with enough O_2 to completely support aerobic respiration. Pezeshki *et al.* (1991) in their study of the cortex structure and metabolic responses of *S. patens* to soil redox conditions arrived at the same conclusion. It is imperative to appreciate the fact that O_2 demands of roots and

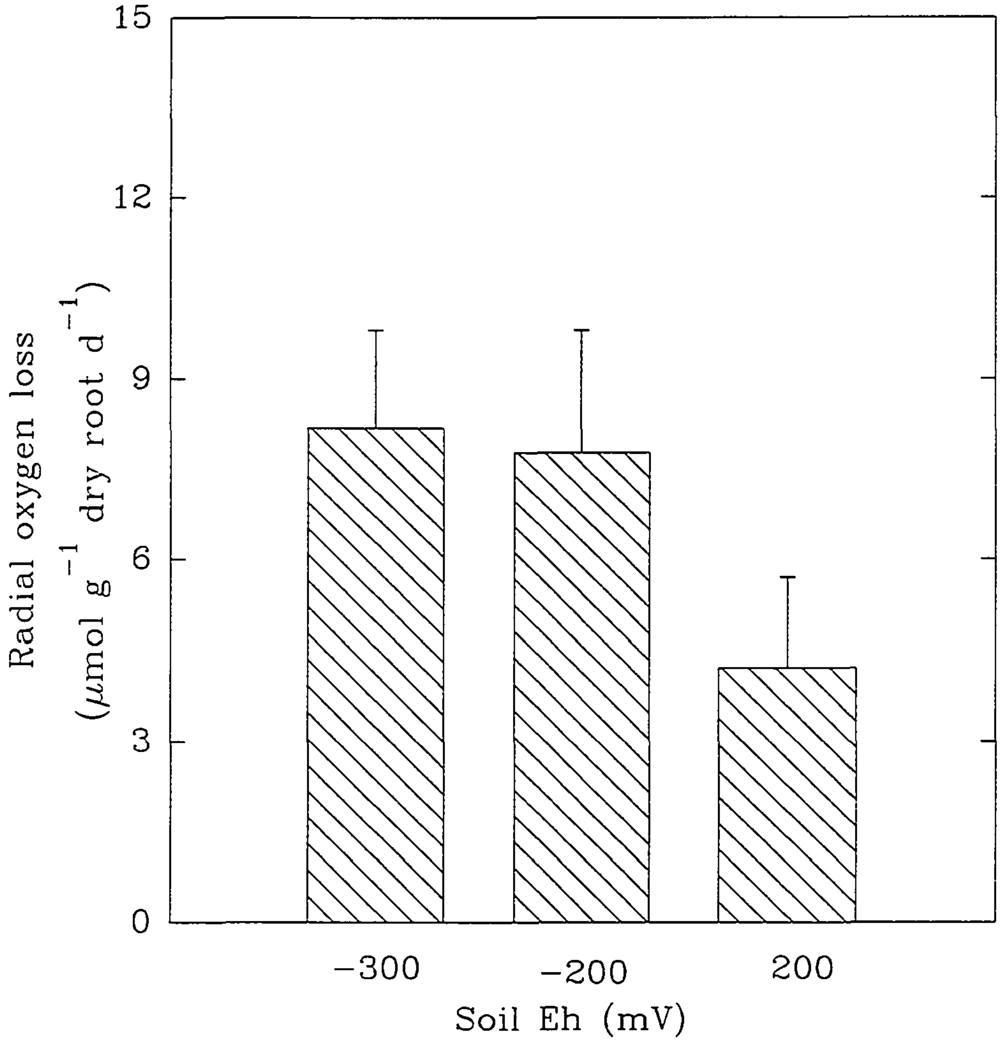


Figure 4.2: Radial oxygen loss (ROL) from *Spartina patens* at the end of the experimental period of 50 days.

rhizosphere are competitive (Armstrong and Beckett, 1987; Armstrong *et al.*, 1991). Roots do not simply release O_2 which is surplus to their environment. Considering the fact that O_2 demand is strong in reduced soils (DeLaune and Pezeshki, 1991), it is likely that much of the O_2 transported from the atmosphere into the plant must have ended up in the soil where it was used for oxidation of reduced chemical species, including CH_4 gas. The likelihood of O_2 conductance to the rhizosphere at the expence of root oxygenation may partly explain why root and shoot growth were adversely inhibited with increase in the intensity of soil Eh. Oxygen supply is essential for root elongation in both flood-tolerant and intolerant plants (Atwell *et al.*, 1985).

Methane production and emission

Figure 4.3 shows methane production with its corresponding diurnal emissions in response to soil reduction intensity and sampling time. Production rates were positively related to soil Eh; the higher the intensity of reduction, the higher the amount of CH_4 produced. This compares favorably with the results on rice (Chapter 3). However, sampling time variations existed. Maximum CH_4 production occured at -300 mV on day 40 (>7900 ng pot^{-1} d^{-1}) but declined to about 7000 ng pot^{-1}d^{-1} at the same soil Eh 10 days later. The boost in CH_4 production by day 40 could be linked to the provision of extra carbon from plant roots in the form of exudates, litter and autolysis products at this stage of growth of the plant. Thereafter, production declined again probably as a result of the dissipation of the extra carbon, and/or inhibition of methanogenesis as a result of reoxidation of reduced chemical species such as sulfides by O_2 supply through the well-developed aerenchyma system. Freney *et al.* (1982) suggested that reoxidation of sulfides to sulfates in the rhizosphere could affect CH_4 production. Schutz *et al.* (1989) reported that methanogenic bacteria are active only under anoxic, reduced soil conditions where there is a high input of labile organic material. Recent reports by Wang *et al.* (1993)

and Masscheleyn *et al.* (1993) showed that CH_4 formation in flooded soils did not occur until the Eh fell below -150 mV. Our results reaffirmed the dependency of CH_4 production on the intensity of soil Eh. Although some CH_4 was detected at 200 mV, methanogenesis was stimulated only at soil Eh levels at or below -200 mV in the presence of organic substrates.

Methane emissions in light varied among Eh treatments and sampling time, but showed a pattern corresponding to CH_4 production on days 20 and 30. For example, the highest emissions at -300mV occured on day 30 when production was equally high and POR attained its maximum level. The variations in CH_4 emissions at the different sampling times may be caused by O_2 availability in the rhizosphere and also by the availability of plant-originated substrates as suggested by Schutz *et al.* (1989). According to Sand-Jensen *et al.* (1982), changes in O_2 availability in the rhizosphere could be caused by changes in the transport of O_2 to the roots, in O_2 consumption by root respiration, possibly by CH_4 oxidation within the plant, and in O_2 consumption by aerobic microorganisms in the rhizosphere. Less O_2 in the rhizosphere could cause a reduction in the efficiency of CH_4 oxidation and an overproportional emission of CH_4 (Conrad, 1989); the converse would also be true leading to lower emissions. Methane fluxes are mostly determined by the processes of methanogenesis and methanotrophy and also by the process of CH_4 transport from the soil into the atmosphere (Conrad, 1993).

In studies by Bartlett *et al.* (1985), CH_4 fluxes have been correlated with CH_4 concentrations in the porewater, indicating that CH_4 fluxes increased with increased production in anaerobic environments. This was consistent with our results. In accord with reports by other researchers (Dacey and Klug, 1979; Chanton *et al.*, 1989), the plant transport system appeared to play a major role in CH_4 fluxes in our study. For example, although CH_4 production at -300 mV on days 20 and 30 was not significantly different ($p<0.05$), emissions were highest on day 30,

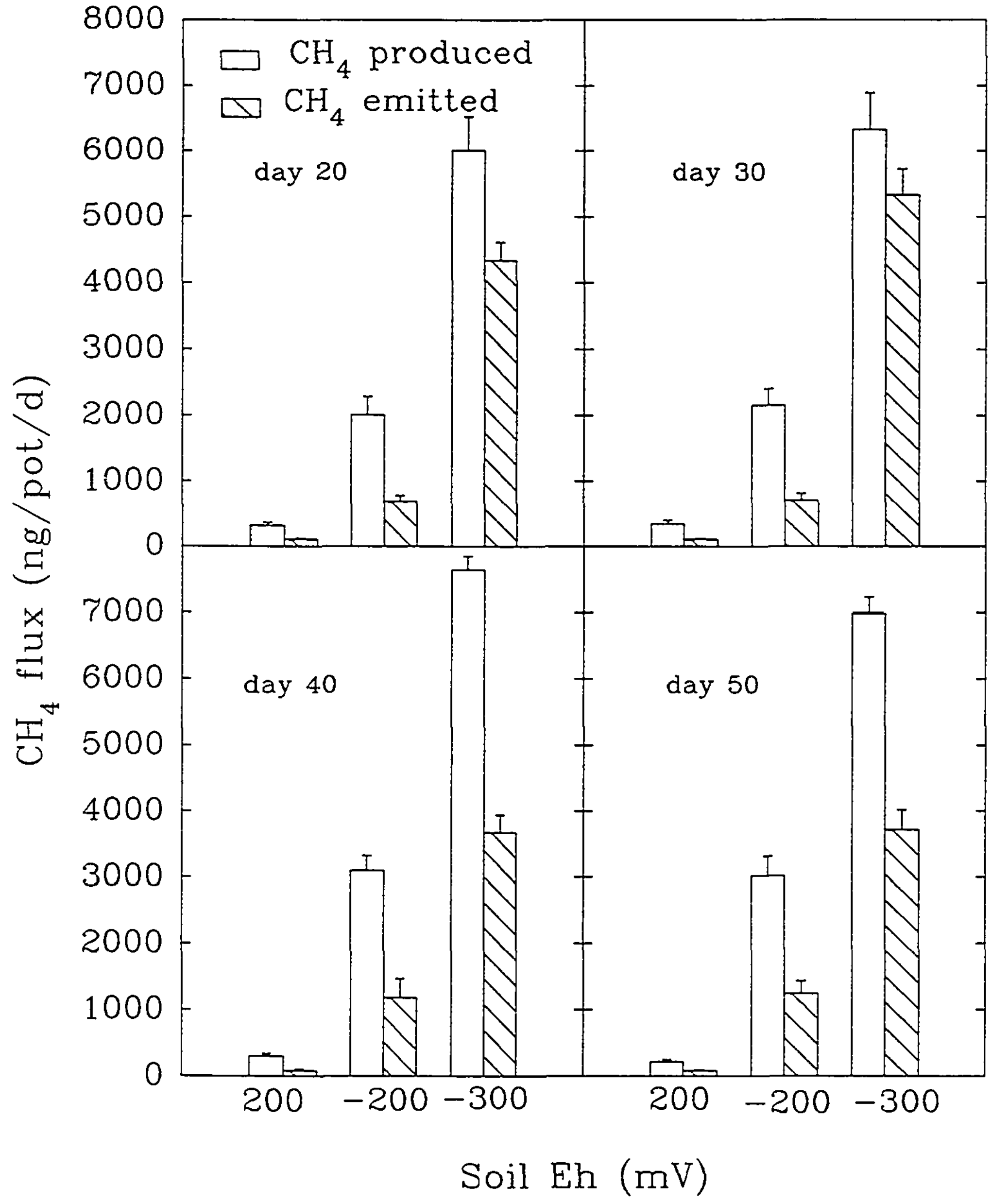


Figure 4.3: Methane production and day-time emission rates from *Spartina patens* as a function of soil Eh at of 20, 30, 40 and 50 days.

corresponding to the time when the plants acquired their maximum air space. Similarly, the fact that CH_4 emission/production ratio started to decline by day 40 was evidence of plant mediated influence on emissions. Since CH_4 emission is the difference between production and oxidation, it appears CH_4 consumption became enhanced at this stage (Fig. 4.1 and Fig. 4.3).

Sass *et al.* (1990) have shown a linear relationship between CH_4 emission from *Oryza sativa* and temporal variations in above ground biomass over a growing season. A linear relationship between plant biomass and CH_4 emission was also noted by (Whiting *et al.* 1991) who reported that this may be related to a combination of plant-enhanced transport and/or substrate production. Our results indicated that although plant biomass generally decreased with intensity of soil reduction, there was no significant difference in total plant biomass between treatments at -200 mV and at -300 mV (day 50), albeit the differences in CH_4 emission rates. This suggests that percent root air-space (Table 4.2), compared to plant biomass, was the more important plant-related factor that controlled net CH_4 emissions in *S. patens.* It has been established that in vegetated sediment/soils, rooted macrophytes such as *Oryza sativa, Nuphar luteum* and *Typha latifolia* are responsible for the majority of CH_4 emissions (Cicerone *et al.*, 1983; Dacey and Klug, 1979; Sebacher *et al.*, 1985). Recently, Whiting *et al.* (1991) reported that more than 90% of the CH_4 released from subtropical grassland is due to transport through plants; the rest was presumably transported by the processes of molecular diffusion and bubble ebullition. Diffusion and ebullition accounted for over 90% of the emissions from unvegetated sediments (Chanton *et al.*, 1989; Kelley *et al.*, 1990). These results were obtained from field studies under natural conditions. The system used in our study involved keeping the soil under continuously stirred suspension. Thus, neither CH_4 emissions by ebullition, soil-water interface diffusion, nor CH_4 entrapment in the soil was likely to influence emissions.

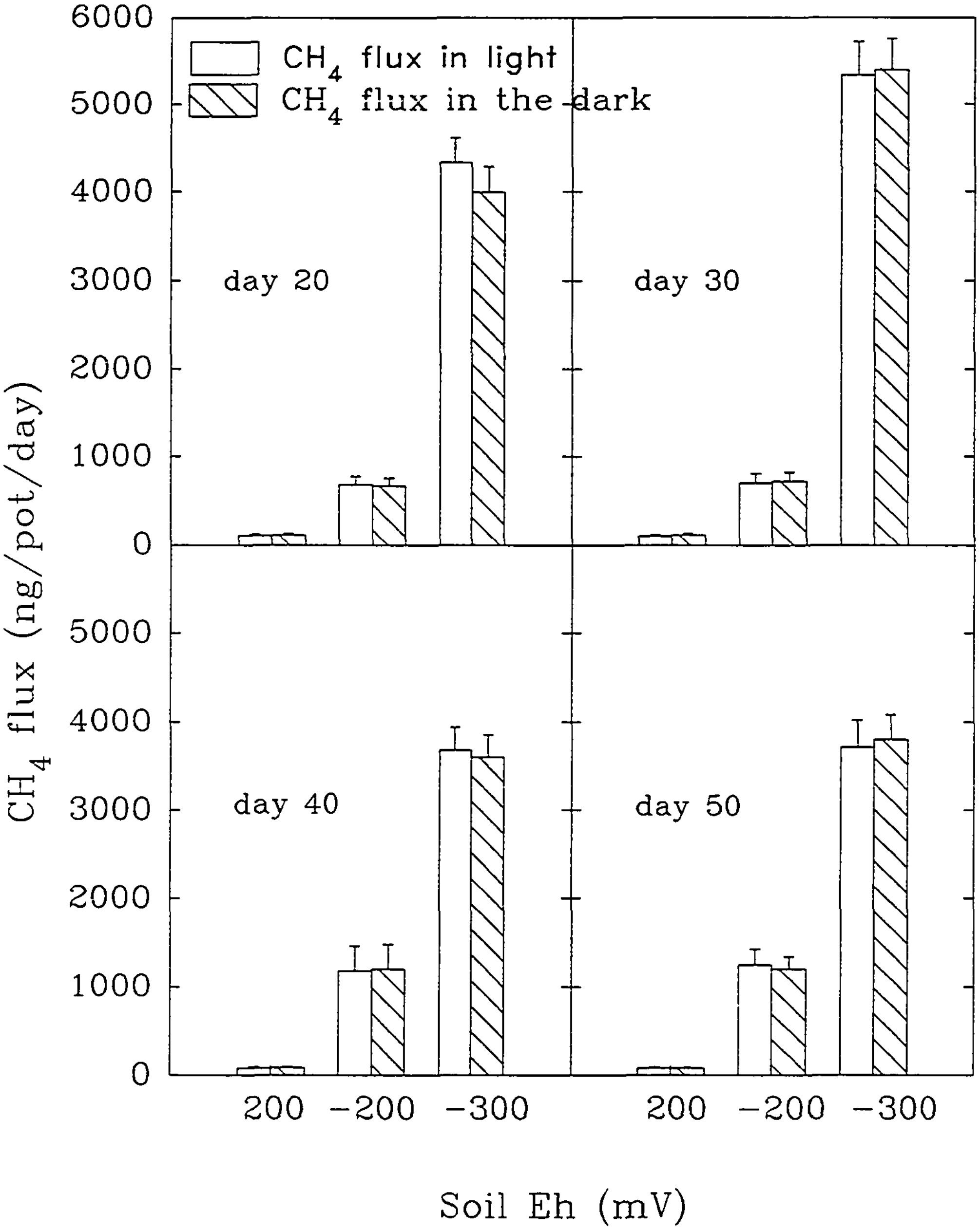


Figure 4.4: Comparison of methane emission rates from *Spartina patens* at day-time and in the dark, as a function of soil Eh at gas sampling days of 20, 30, 40 and 50 days.

No significant differences were detected between CH_4 emission measurements at photon flux density of (1000-1200 μmol m^{-2} s^{-1}) and measurements in the dark (Fig. 4.4). Cicerone *et al.* (1983) and Seiler *et al.*, (1984) found no change in the rate of CH_4 emissions from day to night in rice paddies which they saw as an indication that the CH_4 release was independent of photosynthesis and/or stomatal width of rice. Similar results were found in *Spartina alterniflora* marsh by Bartlett *et al.* (1987) and in *Cladium* marsh by Whiting *et al.* (1991). These findings are consistent with our results and suggest that stomatal dynamics may not be an important factor in CH_4 emissions from *S. patens*. Gas transport through aquatic macrophytes may operate either by molecular diffusion (Armstrong, 1979) or by a thermally pressurized system (Dacey, 1981; Schroder, 1989). Whereas plants with pressurized ventilation exhibit diel variation in CH_4 emission, plants that transport their gas by molecular diffusion do not (Chanton and Dacey, 1991). Our methane measurements in the light and dark (Fig. 4.4) showed no variations, thus suggesting that *S. patens* may not operate a pressurized ventilation system.

Methane oxidation

The effectiveness of CH_3F as a specific inhibitor of CH_4 oxidation by methane monooxygenase enabled us to estimate *in situ* CH_4 oxidation by *Spartina patens* plants. There was a consistently higher CH_4 efflux from CH_3F treated flasks indicating the effectiveness of the inhibitor (Table 4.3).

Methane emissions from planted treatments were higher than those without plants, showing the influence of plants on CH_4 generation and efflux from the soil. The difference in CH_4 emissions between CH_3F treated and untreated flasks was expressed as percentage of produced CH_4 that was oxidized. There was a clear indication that planted flasks had higher CH_4 oxidation than unplanted ones; this was by a factor of 2.6 at -200 mV and 2.8 at -300 mV. The fact that higher oxidation

Table 4.3: Effect of *Spartina patens* and CH_3F on methane production, oxidation and emission at (a) -200mV, and (b) -300mV. Data were collected after 40 d of soil incubation and plant growth. Values are means of 3 replicates, and % CH_4 oxidized was based on production rate.

Treatment	CH_4 produced	CH_4 emitted	CH_4 oxidized	% CH_4 oxidized
	ng flask^{-1} d^{-1}			
		(a) -200 mV		
Planted (+CH_3F)	315.0[1]	309.0	199.0	63.2
(-CH_3F)		110.0		
Fallow (+CH_3F)	308.0	306.0	108	35.1
(-CH_3F)		198.0		
		(b) -300 mV		
Planted (+CH_3F	786.0	765.0	415.0	53.0
(-CH_3F)		350.0		
Fallow (+CH_3F)	772.0	772.0	228.0	29.5
(-CH_3F)		544.0		

[1] All CH_4 production rate measurements were made without CH_3F treatment.

rates occured at -200 mV than at -300 mV could be attributed to the disparity between O_2 conductance and CH_4 production rates at these 2 Eh levels: whereas no significant differences in O_2 leakage from roots was found between treatments (Fig.4.2), CH_4 production at -300 mV was significantly higher than at -200 mV. In effect, there was likely to be relatively more O_2 available for consumption by the methanotrophs at -200 than at -300mV. Although handicapped by the limited number of replicates which could affect the precision of our results, a t-test showed no significant differences ($p<0.05$) between CH_4 oxidation rates in the dessicators (assumed to be the difference between CH_4 production and emission rates) and the direct CH_4 oxidation estimates with CH_3F. Our assumption that the difference between CH_4 production and emission in the desiccator flasks corresponded to the amount oxidized was therefore considered valid. Our percentage CH_4 oxidation values ranged between 53 and 63. These values are lower than those reported by Seiler *et al.* (1984) and Schutz *et al.* (1989) from their paddy studies and suggest that under the same growth conditions, *Oryza sativa* plants may be better conductors of O_2 into the rhizosphere than *Spartina patens*.

CONCLUSIONS

Our study reaffirmed the dependence of net CH_4 efflux from vegetated ecosystems on soil redox intensity in interaction with the physiology of the *S. patens* plant, particularly the percentage root air-space. The intensity of soil Eh induced a concomittant increase in both CH_4 production and root air-space formation, culminating in either the enhancement or inhibition of CH_4 emissions, depending on the age and stage of development of *S. patens* plants. Root and shoot growth, and hence total plant biomass were significantly inhibited by low soil redox conditions. This suggests that despite the existence of root oxygenation mechanism in *S. patens*, there may be lack of adequate O_2 supply to the living root tissues for respiration and nutrient absorption needed for growth. *Spartina patens* grows under flooded conditions in marshlands where it is subjected to low sediment Eh that could influence the survival and competitive ability of this species. Its inability to colonize frequently flooded marsh habitats dominated by *S. alterniflora* in certain coastal marshes has been attributed in part to the lesser ability of *S. patens* for root oxygenation.

As in *Oryza sativa*, *Spartina alterniflora* and *Cladium jamaicense*, CH_4 emissions from *S. patens* were not found to be coupled to stomatal dynamics; neither is this species likely to exhibit a pressurized ventilation system. The use of CH_3F in our CH_4 oxidation study enabled us to successful quantify the amount of CH_4 oxidized at a particular soil Eh by *S. patens* and validated our assumption that CH_4 consumption rate in our microcosms was the difference between the production and emission rates. The study also demonstrated the role played by plants in CH_4 production and emission. However, the level of continuous anaerobiosis and suspension of the soil, as used in our experiments, does not represent the natural environment of our test plant. Methane emission and consumption are highly sensitive to a wide range of biotic and abiotic factors. For example, apart from soil redox status, other factors such as salt and sulfur contents of the natural habitat (brackish marshes) of this species may influence its growth and gas exchange. The results of this study cannot therefore be extrapolated to cover the wide diversity of environmental conditions observed in the field; they however provide a theoretical basis for understanding the influence of soil Eh on the growth, root porosity changes CH_4 production and gas exchange in *Spartina patens*.

REFERENCES TO CHAPTER 4

Armstrong, W. 1979. Aeration in higher plants. pp.697-708. *In*: H.W. Woodhouse(ed.) Ad. Bot. Res.

Armstrong, W., and P.M. Beckett. 1987. Internal aeration and the development of stelar anoxia in submerged roots: A multi-shelled mathematical model combining axial diffusion of oxygen in the cortex with radial losses to the stele, the wall layers and the rhizosphere. New Phytol. 105:221-245.

Armstrong, W., P.M. Beckett, S.H.F.W. Justin, and S. Lythe. 1991. Modelling and other aspects of root aeration. Plant life Under oxygen stress (eds. M.B. Jackson, D.D. Davies and H. Lambers), pp. 267-282. SPB Academic Publishing bV, The Hague.

Atwell, B.J., C.J. Thomson, H. Greenway, G. Wards, and I. Waters 1985. A study of the impaired growth of roots of *Zea mays* seedlings at low oxygen concentrations, Pl. Cell Envir. 8:179-188.

Bartlett, K.B; R.C. Harris, and D.I. Sebacher. 1985. Methane flux from coastal salt marshes. J. Geophy. Res. 90(D3):5710-5720.

Bartlett, D.S. Bartlett, R.C. Harris, and D.I. Sebacher. 1987. Methane emissions along a salt marsh salinity gradient. Biogeochem. 4:183-202.

Blake, D.R., and F.S. Rowland. 1988. Continuing world wide increase in tropospheric methane from 1978 to 1987. Science 239:1129-1131.

Burdick, D.M., and I.A. Mendelssohn. 1990. Relationship between anatomical and metabolic responses to soil waterlogging in the coastal grass *Spartina patens*. J. Exp. Bot. 41(233):233-228.

Chanton, J.P., and J.W.H. Dacey. 1991. Effects of vegetation on methane flux, reservoirs and carbon isotopic composition. *In*: Trace gas emission by plants. (Ed.) T.S Sharkey, E. Holland and H.Mooney. Academic. San Diego, CA. pp. 65-91.

Chanton, C.S. Martens, and C.A. Kelley. 1989. Gas transport from methane saturated tidal freshwater and wetland sediments. Limnol. Oceanog. 34:807-819.

Cicerone, R.J., J.D. Shetter, and C.C. Delwiche. 1983. Seasonal variation of methane flux from a California rice paddy. J. Geophys. Res. 88:11022-11024.

Conrad, R. 1989. Control of methane production in terrestrial ecosystems. *In*: Andreae M.O. and D.S. Schimel (Eds). Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere. Dahlem Konferenzen. pp. 39-58. Wiley, Chichester.

Conrad, R. 1993. Mechanisms of controlling methane emission from wetland rice fields. *In*: Oremland R.S. (ed). The biogeochemistry of global change; Radiative Trace Gases (In Press). Chapman & Hall, New York.

Dacey, J.W.H. 1981. Pressurized ventilation in the yellow water lily. Ecol. 62:1137-1147.

Dacey, J.W.H., and M.J. Klug. 1979. Methane eflux from lake sediments through waterlillies. Science 203:1253-1255.

DeLaune, R.D., and S.R. Pezeshki. 1991. Role of soil chemistry in vegetative ecology of wetlands. Trends in Soil Science 1:101-113.

Drew, M.C., P.H. Saglio, and A. Pradet. 1985. Larger adenylate energy charge and ATP/ADP ratios in aerenchymatous roots of *Zea mays* in anaerobic media as a consequence of improved internal oxygen transport. Planta 165:51-80.

Freney, J.R., V.A. Jacq, and J.F. Baldensperger. 1982. The significance of the biological sulfur cycle in rice production. *In*: Microbiology of tropical soils and plant productivity (I.R. Dommergues and H.G. Bien, eds.), pp. 272-313. Martinus Nijhoff/ Dr. W., The Hague.

Gleason, M.L., and J.C. Zieman. 1981. Influence of tidal inundation on internal oxygen supply of *Spartina alterniflora* and *Spartina patens*. Estuarine Coastal Shelf Sci. 13:47-57.

Holzapfel-Pschorn, A., R. Conrard, and W. Seiler. 1986. Effects of vegetation on the emission of methane from submerged paddy soil. Plant and Soil 92:223-233.

Jensen, C.R., R.J. Luxmoore, S.D. Van Gundy, and L.H. Stolzy. 1969. Root air-space measurements by a pycnometer method. Agron. J. 61:474-475.

Keeley, J.E. 1979. Population differentiation along a flood frequency gradient: Physiological adaptations to flooding in *Nyssa sylvatica*. Ecological Monographs. 49:89-108

Kludze, H.K., R.D. DeLaune, and W.H. Patrick, Jr. 1993. Aerenchyma formation and methane and oxygen exchange in rice. Soil Sci. Soc. Am. J. 51:386-391.

Luxmoore, R.J., R.E. Sojka, and L.H. Stolzy. 1972. Root porosity and growth responses of wheat to aeration and light intensity. Soil Sci. 113:354-357.

Masscheleyn, P.H., R.D. DeLaune, and W.H. Patrick, Jr. 1993. Methane and nitrous oxide emissions from laboratory measurements of rice soil suspension: effect of soil oxidation-reduction status. Chemosphere 26:251-260.

Pezeshki, S.R., S.W. Matthews, and R.D. DeLaune. 1991. Root Cortex structure and metabolic responses of *Spartina patens* to soil redox conditions. Envir. Exp. Bot. 31:91-97.

Pezeshki, S.R., and R.D. DeLaune. 1990. Influence of sediment oxidation-reduction potential on root elongation in *Spartina patens*. Acta Oecol. 11:377-383.

Sand-Jensen, K. C.Prahl, and H. Stokholm. 1982. Oxygen release from roots of submerge aquatic macrophytes. Oikos 38:349-354.

SAS Institute. 1989. SAS/STAT User's Guide. Release 6.03 ed. SAS Institute Inc., Cary, NC.

Sass, R.L., F.M. Fisher, P.A. Harcombe, and F.T. Ferner. 1990. Methane production and emission from a Texas rice field. Global Biogeochem. Cycles 4:47-68.

Schat, H. 1984. A comparative ecophysiological study of the effects of water logging and submergence in dune slack plants: growth, survival and mineral nutrition in sand culture experiments. Oecologia 62:279-286.

Schroder, P. 1989. Characterization of a thermo-osmotic gas transport mechanism in *Alnus glutinosa* (L) Gaertn. Trees 3:38-44.

Schutz, H., W. Seile, and R. Conrad. 1989. Processes involved in formation and emission of methane in rice paddies. Biogeochem. Cycles 7:33-53.

Sebacher, D.I., R.C. Harris, and K.B. Bartlett. 1985. Methane emissions to the atmosphere through aquatic plants. J. Environ. Qual. 14:40-46.

Seiler, W., A. Holzapfel-Pschorn, R. Conrad, and D. Scharfee. 1984. Methane emission from rice paddies. J. Atmos. Chem. 1:241-268.

Seliskar, D.M. 1988. Waterlogging stress and ethylene production in the dune slack plant, *Scirpus americanus*. J. Exp. Bot. 39(209):1639-1648.

Stolzy, L.H., J. Letey, and T.E. Szuszkicwicz. 1961. Root growth and diffusion rates as function of oxygen concentration. Soil Sci. Soc. Am. Proc. 14:55-61.

Wang, Z.P., R.D. DeLaune, and W.H. Patrick, Jr. 1993. Soil redox and pH effects on methane production in a flooded rice soil. Soil Sci. Soc. Am. J. 51:382-385.

Watson, R.T., H. Rodhe, H. Oeschger, and U. Siegenthaler. 1990. Greenhouse gases and aerosols p. 1-40. In J.T. Jenkins, and J.J. Ephraums (eds.), Climate change, the IPCC scientific assessment. Cambridge University Press, Cambridge.

Webb, J., and M.B. Jackson. 1986. A transmission and cryo-scanning electron microscopy study of the formation of aerenchyma (cortical gas-filled space) in adventitious roots of rice (*Oryza sativa*). J. Exp. Bot. 37:832-841.

Whiting, G.J., J.P. Chanton, D.S. Bartlett, and J.D. Happell. 1991. Relationships between CH_4 emissions, biomass, and CO_2 exchange in a subtropical grassland. J. Geophy. Res. 96(D7):13,067-13,071.

Yamasaki, S. 1987. Oxygen demand and supply in *Zizania latifolia* and *Phragmites australis*. Aquatic Bot. 29:205-215.

Yoshida, S., D.A. Romo, J.H. Cock, and K.A. Gomez. 1976. Laboratory manual for physiological studies of rice. 3rd ed. IRRI. Los Banos, Phillippines.

CHAPTER 5

DIFFERENCES IN GASEOUS EXCHANGE AND WETLAND PLANT RESPONSE TO SOIL REDUCTION INTENSITY AND REDUCTION CAPACITY

INTRODUCTION

Although studies on plant physiological responses to wetland conditions abound, there is hardly any information in the literature to distinguish the differences between plant responses to soil reduction intensity and reduction capacity. Neither is there any information to depict or quantify the capacity of soil reduction and relate such quantification to plant physiological responses. DeLaune *et al.* (1990), using titanium-citrate as a redox buffer in nutrient solution, demonstrated that oxygen demand in the rooting medium is important in evaluating physiological functions of wetland plants. In evaluating responses of wetland plant species to prolonged flooding, the substrate condition in which the plants are grown must be properly quantified. Since O_2 is absent at Eh values of about +350mV and lower (DeLaune and Pezeshki, 1991), lack of O_2 only in the soil medium does not reflect actual conditions in the soil. It is therefore imperative that a distinction is made between plant responses to stress under soil reduction intensity and reduction capacity conditions.

The reduction of the inorganic redox system can be described in both intensity and capacity terms (DeLaune and Pezeshki, 1991). The intensity factor determines the relative ease of the reduction and is normally denoted by the redox potential (Eh). The capacity factor on the other hand denotes the amount of the redox system undergoing reduction and is equivalent to the total amount of electrons accepted by the soil oxidants in microbial respiratory activity. The capacity factor may also refer to the total amounts of labile carbon compounds or total energy sources that are utilized during microbial activity, and is best described in terms of its O_2 equivalent.

In effect, two different soils with the same reduction intensity may differ with respect to the capacity factor because of variations in O_2 demand.

It was hypothesized that since the intensity and capacity factors are two different attributes of the soil reduction process, plants would correspondingly exhibit different responses to a soil with the same intensity at varying levels of capacity. The objectives of this study were the following: (1) to distinguish the differences between sublethal plant responses to well defined conditions of soil reduction intensity and capacity, and (2) determine the effect of soil reduction capacity (increasing O_2 demand) on plant growth, ethylene production, root porosity, radial oxygen loss and CH_4 emissions. Measurement of photosynthetic fixation of CO_2 was used as an index of the degree of plant stress induced by the intensity and capacity factors of soil reduction. Wiregrass *(Spartina patens)* and rice *(Oryza sativa)* were the test plants used in the study.

MATERIALS AND METHODS

Plant propagation and soil incubation.

Propagules of wiregrass were collected from the brackish marshes of Louisiana Barataria Basin and were initially nursed for 21 d in plastic pots filled with commercial potting mix (Jiffy mix plus, Jiffy Products of America Chicago, IL.) in the greenhouse. Rice *(c.v. Rico)* seeds were simultaneously germinated and initially nursed for 21 d in a commercial potting mix/Crowley soil mixture (1:1). After this nursery stage, plantlets/seedlings of about the same height, size and appearance were selected and carefully washed of all soil particles and then transfered to pots containing half-strength nutrient solution as described by Yoshida *et al.* (1976). The plantlets/seedlings were grown in the nutrient solution for 21 extra days under the following laboratory conditions: day and night temperatures of 28 ± 2 °C, and 18-h photoperiod with photon flux density of 1000-1200 μmol m^{-2} s^{-2} at canopy level.

These laboratory conditions were maintained throughout the experiments. The solution was changed twice a week.

Soil suspensions (300 g Mhoon soil + 1500 g H_2O, and 400 g Crowley soil + 1400 g H_2O, for wiregrass and rice respectively), were incubated in the redox control system used in Chapters 3 and 4. Information about the Crowley and the Mhoon soils are provided in Chapters 3 and 4, respectively. Both soils were supplemented with ground rice straw (0.6% by weight) as an extra source of carbon to boost soil reduction processes. At the start of soil incubation, the Eh was reduced gradually by continuously bubbling N_2 gas through the soil suspension while the plants adjusted to the system. Nine plants of uniform height and appearance were transplanted into each desiccator flask (microcosm) at the start of the incubation period. Treatments were imposed after 10 days. The experiment was split into two different studies for each of the two test plants: (1) in order to verify the effect of soil intensity factor on CO_2 fixation (photosynthesis), 4 levels of soil Eh: 100, 0, -100 and -200 mV were maintained in the desiccators that were randomly assigned to the Eh treatments, and (2) plant response to soil capacity factor (increasing O_2 demand) was evaluated in a parallel set of incubated soil suspensions prepared as described above but with the Eh maintained at a constant -200 mV. Soil capacity treatments were imposed by application of granular D-glucose (Mallinckrodt Inc., Paris, Kentucky) at the following rates, expressed as % of the soil suspension in each desiccator: 0.08, 0.17 and 0.33 %, considered as treatments 2, 3 and 4 respectively. The control (treatment 1), had no supplementary glucose. Glucose was split-applied over 2, 4 and 6 d in treatments 2, 3 and 4, respectively. Each of the two experimental studies for each test plant species was repeated to allow for statistical analyses of data. The experiment on wiregrass preceded that of rice under the same laboratory conditions.

Measurements

CO_2 fixation

The gaseous exchange chamber technique (Smith *et al.*, 1981) was used to quantify the relative CO_2 fixation as a plant stress indicator, in response to treatments. Basically, the procedure involved placing light and dark chambers (similar to chambers used to sample CH_4 (Kludze *et al.*, 1993)) over the plants. The dark chambers were made by completely covering the outer surface of light chambers with aluminum foil. Sampling for CO_2 was done at 5 min intervals for 25 min. Carbon dioxide was determined on Shumadzu Gas chromatotograph equipped with thermal conductivity detector (TCD). Peak area was determined with a computing intergrator (CD111). The rate of CO_2 fixation or respiration was calculated from the equation:

$$F=k(273/T)(V/A)(\Delta c/\Delta t)$$

where F is the rate of CO_2 fixation or respiration (g C m^{-2} h^{-1}); k is a unit conversion factor (0.322) for the calculation of CO_2 flux as g CO_2-C m^{-2} h^{-1}; T is the temperature of the air within the chamber (K); V is the volume of air within the chamber (L); A is the internal cross-sectional area of the chamber (cm^2) and $\Delta c/\Delta t$ is the rate of change in the concentration of CO_2 in the air within the chamber (μL L^{-1} CO_2 min^{-1}). The value of $\Delta c/\Delta t$ was obtained by using regression analysis to determine the line of best fit. Mean hourly rates of CO_2 gas exchange were expanded to daily rates.

Changes in the CO_2 concentration within the light and dark chambers were considered as estimates of net plant CO_2-C fixation and respiration respectively, and gross CO_2-C fixation was calculated from the algebric sum of the emissions determined with the light and dark chambers. Relative photosynthetic CO_2-C fixation was measured for plants with treatments under both the intensity and capacity factors of soil reduction, 7 and 14 d after the preliminary soil incubation period. Carbon fixation (photosynthesis) under the capacity factor experiment was expressed as a % of the amount fixed in the control (treatment 1), where the control was assigned the value of 100%. All results reported are the mean of 2 fixation and respiration determinations. It must be point out that an important short-coming of this chamber technique in measuring CO_2 fixation is the possibility of some of the CO_2, together with water molecules being adsorbed onto the chamber surface. This could undersetimate CO_2 fixation values.

Soil reduction capacity

Soil reduction capacity was evaluated by determining soil respiration CO_2 and converting the results into O_2 equivalent by stoichiometry. A modified method of soil respiration by Verstraete (1988) was used. Basically, about 50 mL of soil suspension was removed from each desiccator treatment and placed in 500-mL container bottles. Each bottle had a 100-mL beaker containing 10 mL 0.2 N KOH. The soil suspension in each bottle was acidified with HCl to drive dissolved CO_2 into the gas phase. A control bottle had no soil suspension. The bottles were sealed air-tight with non-toxic Rubber Sealant (General Electric, Waterford, NY) and incubated at 20 °C in the dark for 7 days after which the amount of CO_2 released was determined titrimetrically, using phenolfthaleine and methyl orange indicators.

Ethylene production

Ethylene concentration in plant root was determined at the end of the experiment using a modified method descibed in detail by Seliskar (1988). Roots from each treatment were cut into eight 2-cm segments, starting 1 cm from the root tip. The 8-root sections constituted one sample. Plant samples were then placed in 13-mL test tubes and stoppered with rubber serum vial caps. The test tubes were incubated in a water bath at 22 °C for 20 minutes. Accumulated ethylene was determined by withdrawing 1.0 mL gas samples with 2-mL hypodermic syringes. Gas samples were analysed using a Perkin-Elmer 900 gas chromatograph (GC) with a stainless steel column (1.5 m long, 3 mm diameter, HayeSep D, Hayes Separations, Bandera, Tx). Conditions in the GC were as follows: injector, 150 °C; manifold, 150 °C; column temperature program: initial temperature 70 °C raised immediately to 150 °C at 24 °C min^{-1}; carrier gas, N_2 at 30 mL min^{-1}. Root samples were then dried at 65 °C for 48 h to a constant weight. Concentrations of ethylene were calculated as described by Seliskar (1988).

Root porosity was determined by the pycnometer method (Jensen *et al.*, 1969), while radial oxygen loss (ROL) was estimated with the titanium-citrate solution technique as described in detail by DeLaune *et al.* (1990) and Kludze *et al.* (1993). Methane production and emission rates were determined as as decribed earlier (Chapter 3).

Statistical analysis.

Analysis of variance (ANOVA, PROC GLM) of the SAS Statitical package (SAS, 1989) was performed to test for significant differences among treatments (control and the 3 levels of glucose application) for plant physiological changes (shoot ethylene production, root porosity, radial oxygen loss), growth parameters, CO_2 fixation and CH_4 emissions. Mean separation was done with the Duncan's Multiple Range test at the 0.05 level of probability.

RESULTS AND DISCUSSION

CO_2-C fixation responses to soil reduction intensity and capacity.

Figures 5.1a and 5.1b show CO_2 fixation response of wiregrass and rice, respectively, on day 14, to the anaerobic portion of the redox scale, expressed as a percentage. The results indicated that CO_2 fixation response to soil reduction intensity was not significant until the attainment of soil Eh of about -100 mV in both wiregrass and rice. Beyond these two levels, % CO_2-C fixation began to decline significantly until at -200mV when it fell below 50%, indicating a remarkable

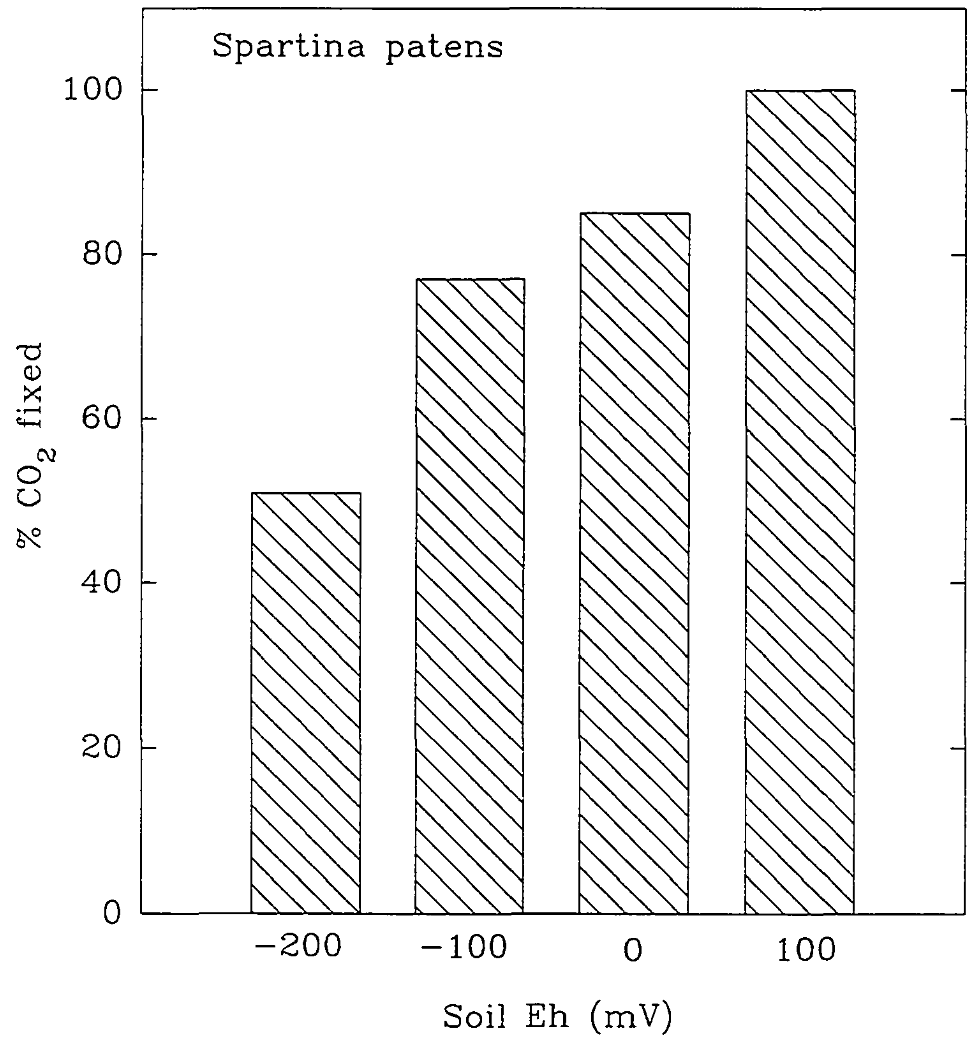


Figure 5.1a: Carbon dioxide fixation in *Spartina patens* as function of soil Eh along the anaerobic portion of the redox scale.

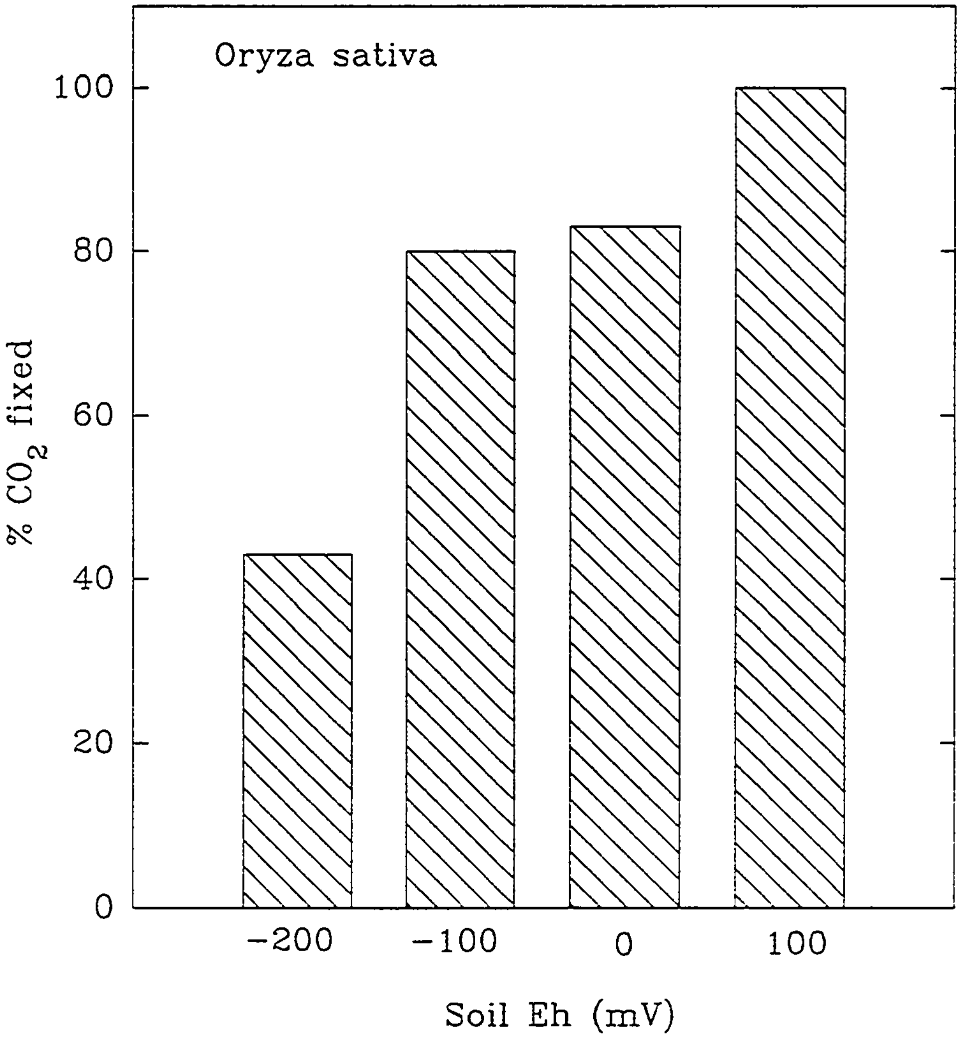


Figure 5.1b: Carbon dioxide fixation in rice as function of soil Eh along the anaerobic portion of the redox scale.

response to the intensity of reduction. When O_2 consumption equivalent of the soil treatments was plotted against % CO_2 fixation at -200 mV (Figs. 5.2a and 5.2b), there was an indication of decreases in relative CO_2 fixation in response to increasing O_2 demand expressed as O_2 consumption equivalent. The higher the O_2 demand, the lower the relative CO_2 fixation. Oxygen consumption equivalent values ranged from 0.6 to 2.6 mg kg^{-1} in wiregrass, and from 0.8 to 2.9 mg kg^{-1} in rice. The control treatment with no supplementary energy source had the least O_2 consumption. Although significant differences in gross CO_2 fixation were established among treatments (Table 5.1), time of sampling differences were not observed.

The reduction in CO_2 fixation with increases in O_2 consumption at the highly reduced Eh of -200 mV suggests plant response to additional stress imposed by increasing O_2 demand and also possibly by accumulated phytotoxins resulting from the reduction process. Among the known documented effects of soil anaerobiosis on plants are early stomatal closure and reduction of photosynthetic rate (Kozlowski and Pallardy, 1979; Pezeshki and Chambers, 1985). DeLaune *et al.* (1990) reported a significant initial reduction in photosynthesis in *Spartina patens* following root exposure to hypoxia. They noted that despite the formation of an extensive aerenchyma system for root O_2 supply from aerial parts, increasing adverse anaerobic conditions could overwhelm the plant's ability to cope.

Physiological responses.

There were no significant differences ($p<0.05$) in root ethylene production among treatments in both the wiregrass and rice cultures (Table 5.2). Ethylene production in waterlogged plants is usually enhanced (Kawase, 1972; Yamamoto and Kozlowski, 1987; Abeles *et al.*, 1992). Ethylene production may be enhanced as a result of reduction in the diffusion of ethylene out of tissue by the floodwater surrounding the tissue (Musgrave *et al*; 1972, Metraux and Kende, 1983), and/or because of increases in ethylene synthesis by flooded tissues (Tang and Kozlowski,

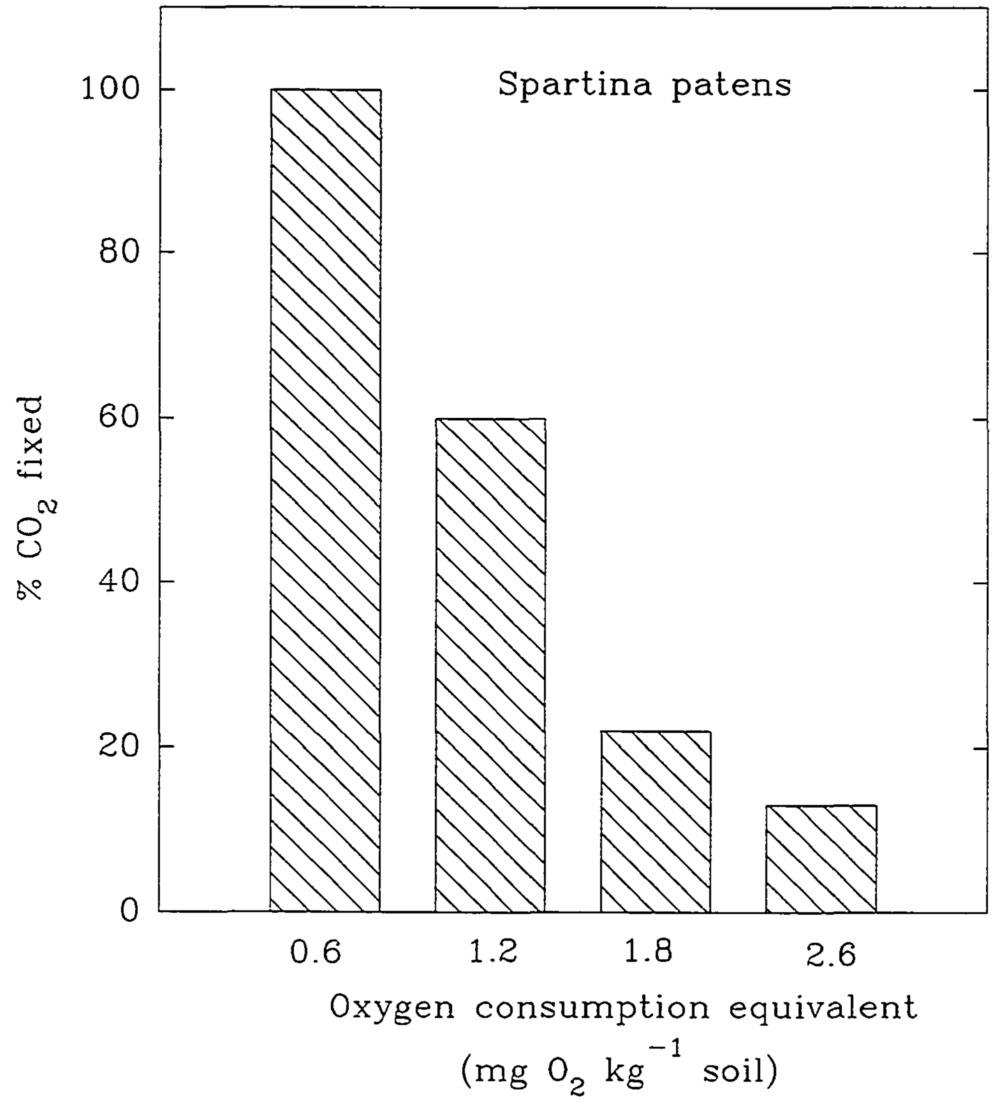


Figure 5.2a: Decreases in CO_2 fixation in *Spartina patens* as a result of increasing soil reduction capacity by adding extra energy source.

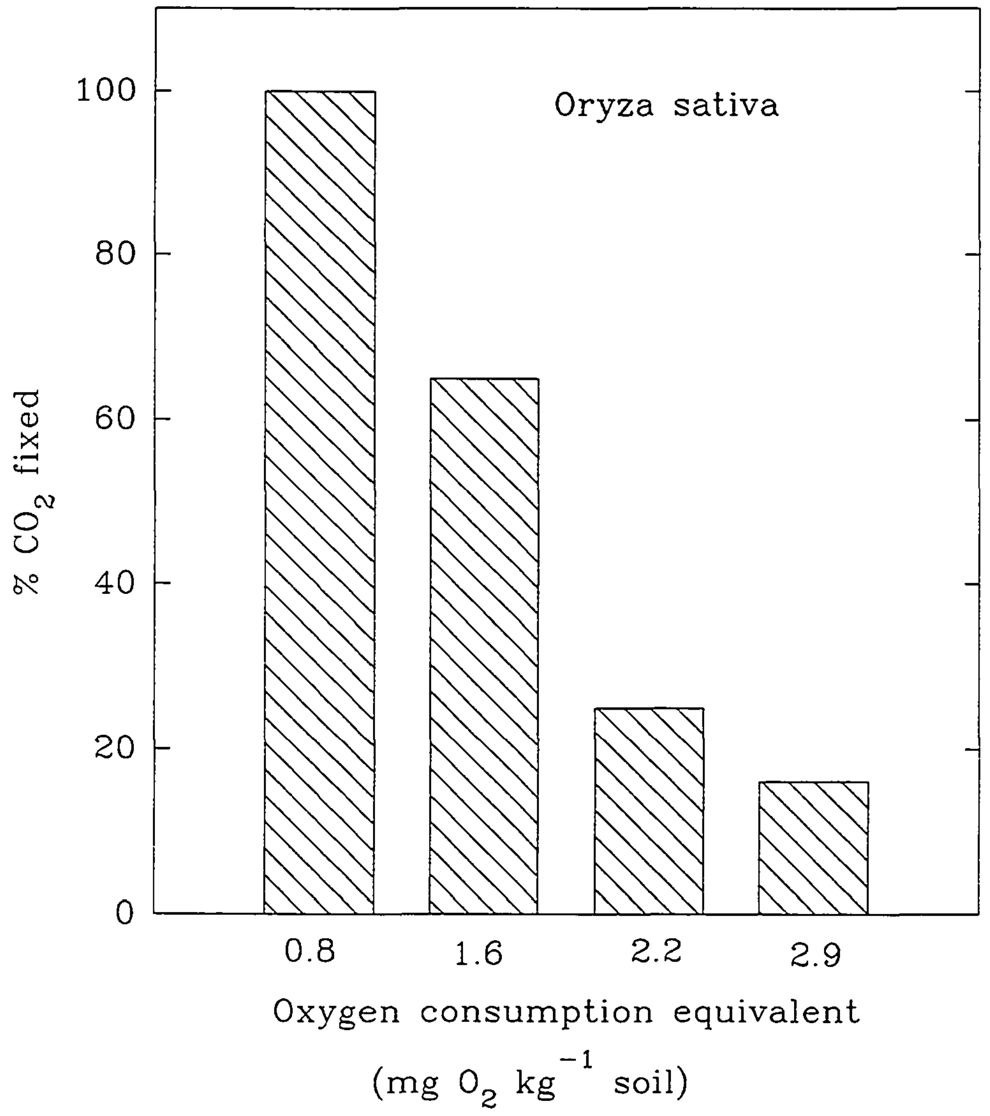


Figure 5.2b: Decreases in CO_2 fixation in rice as a result of increasing soil reduction capacity by adding extra energy source.

Table 5.1: Effect of soil reduction capacity on gross CO_2-C fixation in wiregrass and rice at 7 and 14 days after preliminary soil incubation of Mhoon soil and Crowley soil respectively. The soils were maintained at the same reduction intensity of -200 mV.

Treatment[1]	Gross CO_2-C fixation (g m^{-2} d^{-1})			
	Day 7		Day 14	
	Wiregrass	Rice	Wiregrass	Rice
1	3.0a[2]	3.3a	3.2a	4.8a
2	1.9b	2.1ab	1.8b	2.6b
3	0.8c	1.2b	0.7c	1.3c
4	0.6c	0.9b	0.6c	1.1c

[1] Treatments 1,2,3 and 4 correspond to 0.0 (control), 0.08, 0.16 and 0.32% glucose applied per pot, respectively.

[2] Using the Duncan's Multiple Range test, values within a column followed by the same letter are not statistically different ($P < 0.05$).

1984; Jackson *et al.*, 1987; Atwell *et al.*, 1988). Under soil reducing conditions, the precursor of ethylene (ACC:1-aminocyclopropane-1-carboxylic acid) is produced in the root tips and transported to other root tissues (outer stele or cortex) and the shoot where it is converted to ethylene (Bradford and Young, 1980; Marissen, 1991).

Methionine is the precursor of ACC. Recently, Wang and Arteca (1992) reported stimulation of ACC in waterlogged roots of *Lycopersicon esculentum*. Similar responses have been observed in other species (Jackson *et al.*, 1985; Atwell *et al.*, 1988; Seliskar, 1988). On the other hand, Seliskar (1988) did not observe any increases in ethylene production in waterlogged *Spartina alterniflora* and *Panicum amarulum*, suggesting plant species-specificity of the response. Lack of further increases in plant ethylene production in our study, in spite of increases in supplementary energy source, suggests an inhibition of the synthesis process at the highly reduced soil Eh (-200 mV) coupled with conditions of increasing O_2 demand. Oxygen availability would be a limiting factor in ethylene synthesis since O_2 is required for the convertion of ACC to ethylene. In an earlier study (unpublished data), we found a linear correlation between glucose application rate and soil ethylene production, in accord with the report of other researchers (Arsher and Frankenberger, 1990; Goodlass and Smith, 1978). We also found enhanced ethylene production with increasing intensity of soil reduction within the slightly reduced (+350 to +200 mV), and the reduced (+150 to -100 mV) portions of the redox scale. However, ethylene production within the highly reduced portion ($\leq$-150 mV) of the redox scale declined appreciably. This pattern of ethylene synthesis may therefore suggest that facultative anaerobes rather than obligate anaerobes may be responsible for ethylene synthesis in the soil. It is therefore likely that increased capacity of reduction at higher redox level such as +100 mV may stimulate ethylene production.

Table 5.2: Root ethylene synthesis in response to soil reduction capacity 14 days after preliminary soil incubation. The soils were maintained at the same reduction intensity of -200 mV. Values, expressed in nL ethylene g^{-1} root dry wt., are means of 8 replicates.

Plant species	Treatment[1]			
	1	2	3	4
Wiregrass	0.38a[2]	0.44a	0.37a	0.35a
Rice	0.28a	0.32a	0.30a	0.33a

---

[1] Treatments 1,2,3 and 4 correspond to 0.0 (control), 0.08, 0.16 and 0.32% glucose applied per pot, respectively.

[2] Values within a row followed by the same letter are not significantly different ($P<0.05$), Duncan's Multiple Range test.

The effects of ethylene on plant growth include inhibition of stem elongation, leaf senescence (Kramer, 1951), aerenchyma formation (Drew *et al.*, 1981; Justin and Armstrong, 1991) and gas exchange responses (Bradford, 1983). Pallas and Kays (1982) attributed the adverse effects of ethylene on plant photosynthesis to stomatal closure while Govindrajan and Poovaiah (1982) attibuted it to non-stomatal (metabolic) effects. On the contrary, Bradford (1983) observed no adverse effects of ethylene on stomatal conductance or photosynthesis of *Lycopersicon esculentum* plants, and therefore concluded that the effects of ethylene on stomatal conductance and CO_2 fixation is species-specific. Since we did not find any significant differences in ethylene production in response to soil capacity treatments while differences existed among treatments for CO_2 fixation, it is suggested that ethylene probably played no major role in causing the observed differences in CO_2 fixation among treatments.

Although we earlier found increases in root porosity with increasing soil reduction intensity (+200 to -300 mV) (Chapters 3 and 4), increases in labile soil carbon (Table 5.3) did not significantly enhance root porosity (in agreement with the results in Chapter 2). In essence, it appears soil reduction capacity at the intensity level studied (-200 mV) does not promote further increases in root porosity in these two plant species. Under such a condition, inadequate supply of O_2 to sustain aerobic respiration in plant roots may occur, leading to an induction of alcohol dehydrogenase (ADH) activity, which has been used as an indicator of O_2 deficiency and anaerobic metabolism in plants for survival (Burdick and Mendelssohn, 1987; Naidoo *et al.*, 1992). Alcohol dehydrogenase catalyzes the reduction of acetaldehyde to ethanol during anaerobic respiration (Crawford, 1967; John and Greenway, 1976), thereby ensuring continued generation of energy. Stimulation of ADH under stressful anaerobic conditions has been shown in many wetland plants including rice (John and Greenway, 1976), and *Spartina patens* (Burdick and Mendelssohn, 1990). Lack of

Table 5.3: Root porosity (POR) and radial oxygen loss (ROL) in wiregrass and rice in response to changes in soil reduction capacity, 14 d after preliminary soil incubation. The soils were maintained at the same reduction intensity of -200 mV. Values are means of 6 replicates.

Plant culture	Treatment[1]	% POR	ROL[2]
Wirgrass in	1	25a[3]	7.5a
Mhoon	2	28a	7.0a
soil	3	28a	6.8ab
	4	27a	5.2b
Rice in	1	26a	9.5a
Crowley	2	28a	9.2a
soil	3	30a	7.1a
	4	32a	5.0b

---

[1] Treatments 1,2,3 and 4 correspond to 0.0 (control), 0.08, 0.16 and 0.32% glucose applied per pot, respectively.

[2] Values are expressed in μg g-1 dry shoot d^{-1}.

[3] Using the Duncan's Multiple Range test, values within a column followed by the same letter are not significantly different, ($P<0.05$).

further root air-space formation (root porosity) and synthesis of ethylene in response to increasing O_2 demand suggests that these two parameters may be positively correlated in rice and wiregrass under the experimental conditions It is however possible that root air-space may be enhanced with increase in capacity of reduction at a more elevated intensity of reduction (e.g. +100 mV). Additional studies should address such comparisons. Our data on radial oxygen loss showed a range from 5.2 to 7.5, and from 5.0 to 9.5 μmol O_2 g^{-1} root dry weight d^{-1}, in wiregrass and rice respectively (Table 5.3). In both plant species, O_2 release was reduced by over 33 % in treatment 4 compared to the control. The decreased O_2 release rate with increase in soil labile carbon content was therefore not dependent on root air space which we found to be insignificant among the soil redox capacity treatments. Oxygen demand of roots and rhizosphere are competitive (Armstrong and Beckett, 1987; Armstrong *et al.*, 1991). Oxygen demand, quantified as O_2 consumption equivalent (Fig. 5.2), increased with increase in glucose application. One would therefore expect the higher O_2 releases with increase in O_2 demand. However, this expectation was not realized since ROL decreased with increase in O_2 consumption equivalent. It is suggested that the decreases in ROL in response to increasing O_2 demand occured because plant roots were overwhelmed by the stressful effects of potential phytotoxins such as organic acids, Fe^{2+}, Mn^{2+} and H_2S. Joshi *et al.* (1975) reported that O_2 release from young seedlings of rice was inhibited when the plants were exposed to H_2S concentration of 0.2 mg L^{-1} which limited the ability of the roots to oxidize the rhizosphere.

Growth responses

Root and shoot growth, in both test plants, were severely inhibited by increasing soil capacity, expressed as O_2 consumption equivalent (Table 5.4). Compared to the control, root dry weight decreased by 49 % and 44 % in wiregrass and rice, respectively, in treatment 4. Similarly, shoot dry weight decreased by 37%

Table 5.4: The response of some plant growth parameters of wiregrass and rice to soil reduction capacity, 14 d after preliminary soil incubation. The soils were maintained at the same reduction intensity of -200 mV. Values are means of 3 replicates.

Plant culture	Treatment[1]	Shoot dry wt.	Root dry wt.	Shoot:root ratio
		mg plant^{-1}		
Wiregrass in	1	579.5a	48.1a[2]	12.0c
Mhoon	2	558.1b	39.7b	14.1bc
soil	3	383.5c	25.5c	15.0b
	4	366.0d	21.4d	17.4a
Rice in	1	598.0a	69.4a	8.6a
Crowley	2	573.5a	62.8a	9.1a
soil	3	493.8b	42.0b	11.8b
	4	457.0b	38.1c	12.0c

---

[1] Treatments 1,2,3 and 4 correspond to 0.0 (control), 0.08, 0.16 and 0.32% glucose applied per pot, respectively.

[2] Using the Duncan's Multiple Range test, values within a column followed by the same letter are not significantly different, ($P<0.05$).

and 24%, respectively. There was also a corresponding reduction in root-shoot ratio (by weight). For example, compared to treatment 1 (no extra energy source), root-shoot ratio in treatment 4 with the highest O_2 demand, decreased by about 25 % in both test plants, indicating a more adverse effect on roots than on the shoot. Similar results were obtained when high levels of rice straw were applied to rice cultivars (Chapter 2). Pezeshki and DeLaune (1990) reported substantial reduction of root elongation at soil Eh below +350 mV and complete cessation of root growth at Eh below -100mV. Reduction in root size as a sink for photosynthate causing feed-back inhibition of photosynthesis, has been reported (Chapin, 1991; Drew, 1990). Pezeshki and DeLaune (1990) suggested that such inhibition of root growth could be due to alterations in translocation of growth regulators.

Oxygen supply is essential for root elongation in both flood-tolerant and flood-intolerant plants (Atwell *et al.*, 1985; Topa and McLeod, 1986). Cobb and Kennedy (1987) reported the dependency of of flood-tolerant rice on O_2 availability, while Pezeshki *et al.* (1990) noted that root growth in wiregrass is dramatically affected by lack of O_2 in the soil. Our data on plant growth suggest that wiregrass is more sensitive to soil reduction capacity than rice. Whereas rice has adapted to high rates of alcoholic fermentation by allowing ethanol diffusion from the roots during periods of anaerobic root metabolism (Bertani *et al.*, 1980), similar adaptational feature has not been reported for wiregrass. This may explain why rice was able to cope with the adverse conditions better than wiregrass, as typified by the CO_2 fixation and plant growth data. A decline in root O_2 availability disrupts the flow of photosynthate from shoot to root (Schumacher and Smucker, 1985) which is necessary to support root respiration (Vartapetian *et al.*, 1978).

Methane production and emissions.

In general, CH_4 production tended to increase with every additional increase in glucose application indicating non-inhibition of methanogenesis at these levels of

Table 5.5: Methane production as affected by increases in soil reduction capacity, through the addition of extra energy source. The soils were maintained at the same reduction intensity of -200 mV. Values are means of 3 replicates.

Treatment[1]	Methane production (ng pot^{-1} d^{-1})			
	Wiregrass		Rice	
	Day 7	Day 14	Day 7	Day 14
1	2110c[2]	2000c	2511c	2480d
2	2485b	2408b	2675b	2670c
3	2508ab	2485b	2773a	2758b
4	2557a	2511a	2788a	2800a

[1] Treatments 1,2,3 and 4 correspond to 0.0 (control), 0.08, 0.16 and 0.32% glucose applied per pot, respectively.

[2] Using the Duncan's Multiple Range test, values followed by the same letter in a column are not significantly different, ($P<0.05$).

Table 5.6: Methane emissions from wiregrass and rice as affected by increases in soil reduction capacity, through the addition of extra energy source. The soils were maintained at the same reduction intensity of -200 mV. Values are means of 2 replicates.

Treatment	Methane emission (ng pot^{-1} d^{-1})			
	Wiregrass		Rice	
	Day 7	Day 14	Day 7	Day 14
1	285b[2]	232a	1758a	1456b
2	296a	219ab	1853a	1545a
3	279c	197b	1543b	1433c
4	262c	159c	1378c	1154d

[1] Treatments 1,2,3 and 4 correspond to 0.0 (control), 0.08, 0.16 and 0.32% glucose applied per pot, respectively.

[2] Using the Duncan's Multiple Range test, values followed by the same letter in a column are not significantly different, ($P < 0.05$).

application (Table 5.5). Increases in CH_4 production is in accord with reports of other researchers (Vermoessen *et al.*, 1991; Yagi and Minami, 1990). Addition of easily decomposable substrates to waterlogged soils can result in an increased accumulation of organic acids which are eventually broken down to CO_2 and CH_4 (Chandrasekaran and Yoshida, 1973). Table 5.6 depicts CH_4 emission rates on days 7 and 14 of plant growth in the incubated soil treatments. Although CH_4 production in both soils increased with increase in application of extra energy source, CH_4 emissions tended to decrease. The significant decreases in emissions, especially in treatment 4, may therefore be linked to the decreases in total plant biomass (Table 5.2) with increasing redox capacity. Whiting *et al* (1991) and Sass *et al* (1990) reported a linear relationship between plant biomass and CH_4 emissions. A reduction in plant biomass results in the curtailment of the total diffusive pathway of CH_4 gas from the soil to the atmosphere.

In conclusion, the results of this study suggest that plants respond differently to soil reduction intensity and capacity. Although the CO_2 fixation of both wiregrass and rice along the anaerobic portion of the redox scale was unaffected until at Eh -100 mV, slight increases in soil reduction capacity resulted in significant reductions in CO_2 fixation. Whereas CO_2 fixation and plant growth, in terms of root and shoot dry weights, were significantly reduced by increasing soil capacity, other plant responses such as root porosity and ethylene synthesis were not significantly affected in both test plants. These results indicate functional similarities between the wiregrass and rice. Plant growth was adversely affected probably because of reductions in CO_2 fixation, possibly resulting from impairment of the photosynthetic apparatus and the dependence of plants on the less efficient anaerobic respiration process to generate energy. Therefore, in order to properly evaluate plant physiological responses to reducing soil conditions, the intensity and capacity factors of soil reduction must be considered.

REFERENCES TO CHAPTER 5

Abeles, F.B., P.W. Morgan, and M.E. Salveit, Jr. 1992. Ethylene in Plant Biology. Academic Press, Inc. San Diego.

Armstrong, W., and P.M. Beckett 1987. Internal aeration and the development of stelaranoxia in submerged roots. A multishelled mathematical model combining axial diffusion of oxygen in the cortex with radial losses to the stele, the wall layers, and the rhizosphere. New Phytol. 102:221-245.

Armstrong, W., S.H.F. Justin, P.M. Beckett, and S. Lythe. 1991. Root adaptation to soil waterlogging. Aquatic Bot. 39:57-73.

Arshad, M., and W.T. Frankenberg, Jr. 1990. Ethylene in Soil Response to Organic Amendments. Soil Sci. Soc. Am. J. 54:1026-1031.

Atwell, B.J., C.J. Thomson, H. Greenway, G. Wards, and I. Waters. 1985. A study of the impaired growth of roots of *Zea mays* seedlings at low oxygen concentration. Pl. Cell Envir. 8:179-188.

Atwell, B.J., M.C. Drew, and M.B. Jackson. 1988. The influence of oxygen efficiency on ethylene synthesis, 1-aminoclopropane-1-carboxilic acid levels and aerenchyma formation in roots of *Zea mays*. Physiol. Plant. 72:15-22.

Bertani, A. I. Brabbilla, and F. Menegus. 1980. Effect of anaerobiosis on rice seedlings: Growth, metabolic rate, and fate of fermentation products. J Exp. Bot. 31:325-331.

Bradford, K.J. 1983. Involvement of plant growth substances in the alteration of leaf gas exchange of flooded tomato plants. Pl. Physiol. 73:480-483.

Bradford, K.J., and S.F. Young. 1980. Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor in waterlogged tomato plants. Pl. Physiol. 65:322-326.

Burdick, D.M., and I.A. Mendelssohn. 1990. Relationship between anatomical and metabolic responses to soil watrlogging in the coastal grass *Spartina patens*. J. Exp. Bot. 41:223-228.

Burdick, D.M., and I.A. Mendelssohn. 1987. Waterlogging responses in dune, swale, and marsh populations of *Spartina patens* under field conditions. Oecologia 74:321-329.

Chandrasekaran, S., and Y. Yoshida. 1973. Effect of organic acid transformations in submerged soils on growth of rice plant. Soil Sc. Plant Nutr. 19:39-45.

Chapin, F.S. 1991. Integrated responses of plants to stress. Biosc. 41:29-36.

Cobb, B.G., and R.A. Kennedy. 1987. Distribution of alcohol dehydrogenase in roots and shoots of rice (*Oryza sativa*) and *Echinochloa* seedlings. Pl. Cell Envir. 10:633-638.

Crawford, R.M.M. 1967. Alcohol dehydrogenase activity in relation to flooding tolerance in roots. J.Exp. Bot. 18:458-464.

DeLaune, R.D., and S.R. Pezeshki. 1991. Role of soil chemistry in vegetative ecology of wetlands. Trends Soil Sci. 1:101-113.

DeLaune, R.D., S.R. Pezeshki, and J.H. Pardue. 1990. An oxidation-reduction buffer for valuating the physiological response of plants to root oxygen stress. Envir. Exp. Bot. 30:243-247.

Drew, M.C. 1990. Sensing soil oxygen. Plant Cell Envir. 18:223-230.

Drew, M.C., M.B. Jackson, and S. Giffard. 1981. Inhibition of silver ions of gas space (aerenchyma) formation in adventitious roots of *Zea mays* L. subjected to exogenous ethylene or to oxygen deficiency. Planta 153:217-224.

Goodlass, G., and K.A. Smith. 1978. Effect of pH, organic matter content and nitrate on the evolution of ethylene from soils. Soil Biol. Biochem. 10:193-199.

Govindrajan, A.G., and B.W. Poovaiah. 1982. Effect of root zone carbon dioxide enrichment on ethylene inhibition of carbon assimilation in potato plants. Physiol. Plant. 55:465-469.

Jackson, M.B. 1985. Ethylene and responses of plants to soil waterlogging and submergence. Ann. Rev. Pl. Physiol. 36:145-174.

Jackson, M.B., I. Waters, T. Setter, and Greenway. 1987. Injury to rice plants caused by complete submergence: A contribution by ethylene (ethene). J. Exp. Bot. 38:1826-1838.

Jensen, C.R., R.J. Luxmoore, S.D. Van Grudy, and L.H. Stolzy. 1969. Root air space measurements by a pycnometer method. Agron. J. 61:474-475.

John, C.D., and H. Greenway. 1976. Alcohol fermentation and activity of some enzymes in rice roots under anaerobiosis. Australian J. Pl. Physiol. 1:513-520.

Joshi, M.M., I.K.A. Ibrahim, and J.P. Hollis. 1975. Hydrogen Sulfide: effects on the physiology of rice plants and relation to straighthead disease. Phytopathol. 65:1165-1170.

Justin, S.H.F., and W.Armstrong. 1991. Evidence for the invovement of ethylene in aerenchyma formation in adventitious roots of rice. New Phytol. 118:49-62.

Kawase, M. 1972. Effects of flooding on ethylene concentration in horticultural plants. J. Amer. Soc. Hort. Sci. 97:584-588.

Kludze, H.K., R.D. DeLaune, and W.H. Patrick, Jr. 1993. Aerenchyma formation and methane and oxygen exchange in rice. Soil Sci. Soc. Am. J. 57:386-391.

Kozlowski, T.T., and S.G Pallardy. 1979. Stomatal responses of *Fraxinus pennsylvanica* seedlings during and after flooding. Physiol. Plant. 46:155-158.

Kramer, P.J. 1951. Causes of injury to plants resulting from flooding of the soil. Plant Physiol. 26:722-736.

Marissen, N., W.A. Kanneworff, and L.H. van der Plas. 1991. Effects of anaerobiosis in ethylene production, respiration and flowering in iris bulbs. Physiol. Plant. 82:465-473.

Metraux, J.P., and H. Kende. 1983. The role ethylene in the growth response of submerged deepwater rice. Plant Physiol. 72:441-446.

Musgrave, A., M.B. Jackson, and E. Ling. 1972. Callitriche stem elongation is controlled by ethylene and gibberellin. Nature New Biol. 238:95-96.

Naidoo, G., K.L. McKee, and I.A. Mendelssohn. 1992. Anatomical and metabolic responses to waterlogging and salinity in *Spartina alterniflora* and *S. patens* (Poaceae). Am. J. Bot. 79(7):765-770.

Pallas, J.E., and S.J. Kays. 1982. Inhibition of photosynthesis by ethene--a stomatal effect. Pl. Physiol. 70:598-601.

Pezeshki, S.R., and R.D. DeLaune. 1990. Influence of sediment oxidation-reduction potential on root elongation in *Spartina patens*. Acta Oecologia 11:377-383.

Pezeshki, S.R., and J.L. Chambers. 1985. Stomatal and photosynthetic response of sweetgum (*Liquidamber styraciflua* L) to flooding. Ca. J. For. Res. 15:371-375.

SAS Institute Inc.1989. SAS/STAT User's Guide , Version 6, Fourth Edition, Vol. 2. SAS Institute Inc., Cary, North Carolina pp. 846

Sass, R.L., F.M. Fisher, P.A. Harcombe, and F.T. Ferner. 1990. Methane production and emission from a Texas rice field. Global Biogeochem. Cycles 4:47-68.

Schumacher, T.E., and A.J.M. Smucker 1985. Carbon transport and root respiration of split root systems of *Phaseolus vulgaris* subjected to short term localized anoxia. Plant Physiol. 78:359-364.

Seliskar, D.M. 1988. Waterlogging stress and ethylene production in the dune slack plant, *Scirpus americanus*. J. Exp. Bot. 39:1639-1648.

Smith, C.J., R.D. DeLaune, and W.H. Patrick. 1981. A method for determining stress in wetland plant communities following an oil spill. Environmental Pollution (Serie A) 26:197-304.

Tang, Z.C., and T.T. Kozlowski. 1984. Ethylene production and morphological adaptation of woody plants to flooding. Can. J. Bot. 62:1659-1664.

Topa, M.A., and K.W. McLeod. 1986. Aerenchyma and lenticel formation in pine seedlings: A possible avoidance mechanism to anaerobic growth conditions. Physiol. Plant. 68:540-550.

Vartapetian, B.B., I.N. Andreeva, I.P. Maslova, and N.G. Davtian. 1970. The oxygen and ultra-structure of root cells. Agrochimica 15:1-19.

Vermoessen, A., H. Ramon, and O. Van Cleemput. 1991. Composition of the soil gas phase. Permanent gases and hydrocarbons. Pedologie 41:119-132.

Verstraete, W. 1988. Biotechnological processes in environmental technology. Part II, 4. Lecture Notes. Laboratory of Microbial Ecology, State University of Gent, Belgium.

Wang, T., and R.N. Arteca. 1992. Effects of low oxygen root stress on ethylene biosynthesis in tomato plants *(Lycopersicon esculentum)*. Pl. Physiol. 98:97-100.

Whiting, G.J., J.P. Chanton, D.S. Bartlette, and J.D. Happel. 1991. Relationships between CH4 emissions, biomass, and CO_2 exchange in a subtropical grassland. J. Geophy. Res. 96(D7):13,067-13,071.

Yagi, K., and K. Minami 1990. Effect of organic matter application on methane emission from some Japanese paddy fields. Soil Sc. Plant Nutr. 36:599-610.

Yamamoto, F., and T.T. Kozlowski. 1987. Effects of flooding, tilting of stems and ethrel application on growth, stem anatomy and ethylene production of *Pinus densiflora*. J. Exp. Bot. 38:293-310.

Yoshida, S., D.A. Forno, J.H. Cock, and K.A. Gomez. 1976. Laboratory manual or physiological studies of rice, 3rd. ed. International Rice Research Institute, Los Banos, Philippines.

SUMMARY AND CONCLUSIONS

There has been an increasing interest in the investigation of plant physiological changes and the emission of the CH_4 in response to wetland conditions. Wetlands are characterized by the saturation of their soils with water, resulting in reducing conditions explained in terms of soil reduction intensity and capacity. Such reducing conditions result in the curtailment of O_2 supply which affects (1) the growth and adaptation of wetland plant species, and (2) the production and emission of CH_4 gas. Although studies on gas exchange and plant physiological functions in response to wetland conditions abound, there is limited information in the literature to relate such responses to well defined levels of soil reduction intensity or the degree of anaerobiosis. Neither is there any information to depict or quantify the capacity factor of soil reduction and relate such quatification to plant physiological responses and gas exchange, particularly CH_4 and O_2 transport. Lack of these vital information has prompted this study. As part of this study, a colorimetric procedure for assaying O_2 released by whole root systems was tested. Methods used by some workers to measure root O_2 release have been based on O_2-depleted solutions which are a poor analogue of natuaral sediments since they do not mimic their high O_2 demand and low redox potential; in effect, results of such procedures underestimate the oxygen transport potential of aquatic plants. Soil/sediment O_2 demand acts as a sink for O_2 released by roots, and the absence of such a sink in nutrient solutions poses a serious defect in estimating released O_2 because any O_2 that is released is predisposed to reabsorption by respiring root tissue.

The first part of the study sought to test a colorimetric technique of assaying oxygen leaked from whole root system of seven rice cultivars. Titanium citrate buffer, which is a strong sink for O_2 and whose color change is proportional to oxygen concentration in a medium, was used for the test. The technique was utilized in estimating the oxidizing power of the test plants, rice (*Oryza sativa*) and wiregrass (*Spartina patens*), in subsequent experiments. Secondly, the response of three rice cultivars to different dosages of rice straw was evaluated. This was followed by studies on the effect of well-defined controlled soil reduction intensity levels on plant growth, root porosity (POR), radial oxygen loss (ROL), and on CH_4 production and emission from the test plants. The final part of this study sought to differentiate between plant responses to soil redox intensity and capacity with respect to growth, CO_2 fixation and gas exchange, particularly CH_4 and O_2 transport.

An experimental technique involving the use of titanium citrate buffer solution was used to assay solution O_2 concentration colorimetrically. The buffer solution is non-toxic and consumes O_2 by first-order redox reaction. The technique was tested for its potential to quantify dissolved O_2 concentration in solution bathing the whole root system of container-grown rice cultivars. Seven rice cultivars were grown in sand culture fertilized with nutrient solution under well drained and hypoxic conditions. The rate of O_2 release was calculated by extrapolation of the measured absorbance to standard curves after placing plant roots in the buffer solution for 6 h. Since the titanium solution provides a good analogue of natural reduced soils, and since Ti^{3+} ions mimic the O_2 sink of such soils by reacting with the O_2 as it is released, the technique provides a very sensitive way of estimating rhizosphere oxidation. It was shown that estimates of radial oxygen loss with the technique were comparable to those obtained with a polarographic oxygen electrode. Variations existed among the seven rice cultivars, between aeration status, and between sampling time for O_2 release rate. Hypoxic treatments had higher releases than those from the drained treatments. Similarly, older plants had higher root oxidation than the younger plants. Oxygen release rates ranged from 10.0 to 18.6 μ mol plant^{-1} d^{-1} in the drained treatments and from 10.0 to 33.2 μmol plant^{-1} d^{-1} in flooded treatments. Influences of aeration status of the root medium on root porosity (POR) were also evaluated and results indicated cultivar and plant age differences. Root porosity was enhanced by hypoxia. Some cultivars exhibited high POR-ROL correlation while others did not. Rapid induction of root air-space formation at early stages coupled with correspondingly high O_2 leakage from roots may explain why some rice cultivars cope with effects of anaerobiosis better than others. For example, a high POR-ROL correlation was found in the cultivars *Rico* and *Lemont* which suggests the ability of these cultivars to tolerate reducing conditions and may explain why they are currently grown successfully in low-land cultivation on a larger scale in Louisiana.

Although the literature has reported effects of exogenous organic matter application on CH_4 emissions from paddy fields, information is lacking on plant-related influences on gas transport when different doses of straw are applied to the soil. In an experiment involving the growth of three rice cultivars under different doses of straw, plant growth was impaired at the application rate of 22 tons ha^{-1}. This in turn resulted in the reduction of CH_4 emissions since the total diffusive pathway of the gas was significantly reduced. Results indicated an interactive effect of cultivar and straw application rate on the transport of gases to-and-from the soil. At the straw application rates in this study, methanogenesis was not inhibited.

Limited information exists on the direct link between the intensity of soil reduction (anaerobiosis) and gas exchange in rice (*Oryza sativa* L). A laboratory experiment was conducted to determine the extent to which specific levels of soil redox potential (Eh) could influence root porosity, O_2 transport, and CH_4 production and emission. Plants were grown under controlled redox intensity levels of 200, -200, and -300 mV, using a Louisiana paddy soil (Crowley silt loam: monmorillonitic thermic Typic Albaqualf). Methane production and its emission increased with decrease in soil Eh. Methane production increased 10-fold, whilst its emission was enhanced 17-fold when soil Eh dropped from -200mV to -300mV. A positive relationship was established between the intensity of soil anaerobiosis and both POR and ROL. Root and shoot dry weights and root length decreased with decrease in soil Eh. Results of this study demonstrated that soil Eh influences net CH_4 flux from rice in two ways: (1) it directly determines the amount and rate of CH_4 production in the soil, and (2) it initiates morphological and physiological changes in the rice plant that affect gas exchange between the soil and the atmosphere. Although the results may not necessarily reflect actual conditions in the field, they provide a theoretical basis for understanding the influence of soil Eh on rice growth, CH_4 production and gas exchange.

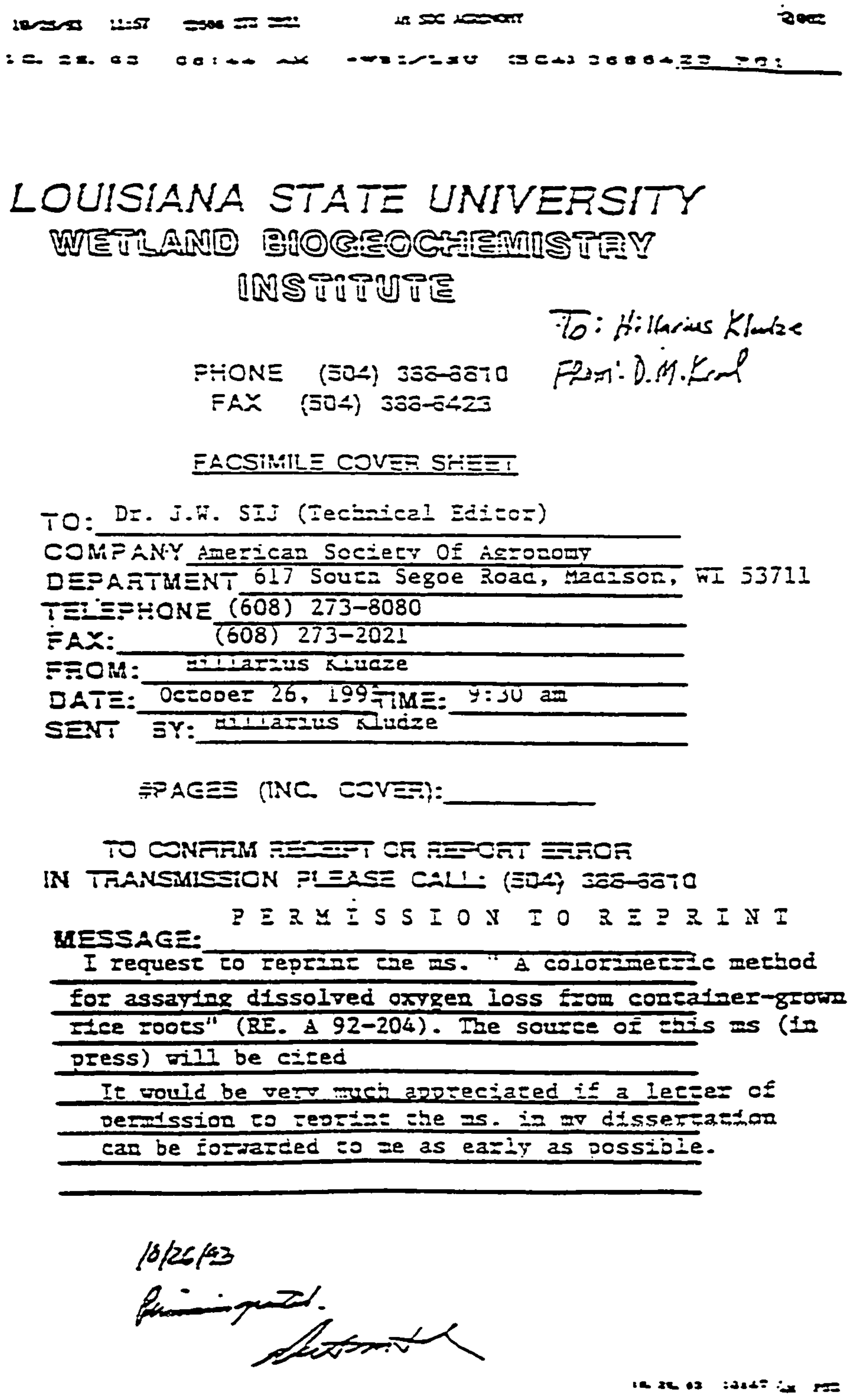
Although the overall budget of atmospheric CH_4 is fairly well established, the strength of individual sources remains uncertain. An understanding of the different ecosystems that control CH_4 dynamics is crucial to the formulation of any mitigation strategies for this greenhouse gas. Laboratory experiments were therefore conducted to determine how soil oxidation-reduction (Eh) intensity could influence the growth, root porosity (POR), radial oygen loss (ROL), and CH_4 production, oxidation and emission in the marshland plant, *Spartina patens* (Ait) Muh (wiregrass). Plants were grown for 50 d in a Mississippi alluvial soil (silty-clay loam, Typic Fluvaquent) under controlled Eh values of 200, -200 and -300mV. Root and shoot dry weights and root length decreased with increase in Eh intensity. Root air-space (porosity) was enhanced by decrease in Eh up to about 30 d when further development levelled off and ceased. It appears root porosity in flooded *S. patens* depends on some chronological event, irrespective of the redox status. This requires further investigation. Methane production was generally enhanced by the intensity of reduction. Emissions were commensurate with production levels but were influenced by plant-related factors, especially POR. No differences ($p<0.05$) existed between emissions in light and in the dark, suggesting lack of stomatal influence. Methane oxidation, estimated by CH_3F, was found to be higher in -200 mV treatments compared to treatments at -300 mV. Results of this study demonstrated that as in *Oryza sativa*, soil redox intensity initiates physiological changes in *S. patens* which in turn modulates the plant's gas transport.

Oxygen demand and redox chemistry of the rooting medium as related to wetland plant response were also examined. *Spartina patens* and *Oryza sativa* plants were grown in a range of controlled soil reduction intensity and capacity conditions in Mhoon silty-clay loam (Typic Fluvaquent) and Crowley silty loam (Typic Albaqualf), respectively. Increasing soil reduction capacity, expressed as O_2 consumption equivalent, was obtained by adding doses of glucose as an extra energy source at a pre-set redox intensity of -200mV. CO_2 fixation responses of both species to intensity and capacity factors in addition to plant responses to reduction capacity in growth, ethylene production, root porosity and CH_4 emissions were measured. CO_2 fixation did not respond to intensity of anaerobiosis until redox values of -100mV in both test plants. Fixation of CO_2 and plant growth were significantly reduced ($p<0.05$) with increasing soil reduction capacity. Oxygen consumption increased linearly with increase in application of extra energy source. At higher glucose application rates, and with time, CH_4 emissions decreased with increase in soil capacity levels (O_2 demand), suggesting a complex relationship between soil reduction capacity and the physiological functioning of plants. Plant ethylene production was unaffected by increases in soil reduction capacity at the low soil Eh of -200 mV. Results of this study indicated that plants may respond differently to the intensity and capacity factors of soil anaerobiosis. Evaluating response of flood-tolerant species to soil/sediment O_2 demand therefore requires proper quantificaton of such conditions in the substrate in which the plants are grown.

Overall, this study has applied an experimental procedure for assaying the rate of O_2 release from whole plant root system. By acting as a strong sink, Ti^{3+} of the titanium-citrate buffer scavenges O_2 as it is released by roots, thus preventing it from being reabsorbed by respiring root tissue. The technique reduces the difficulty in measuring total O_2 exchange by root systems of plants which may be of a greater significance to *in situ* rhizosphere oxidation. Immersion of the whole root system of plants in the titanium-citrate solution which has a high O_2 demand and low Eh, to some extent, mimics the natural medium of wetland plants. Estimates of O_2 concentration by this technique would therefore provide a more accurate measure of the O_2 releasing potential of wetland plants. Apart from establishing an oxidized zhizosphere which may reduce CH_4 emissions and the assimilation of reduced phytotoxins by roots, the process of root O_2 release is essential in constructed wetlands used for waste water treatment, as it may enhance nitrification and reduce total O_2 demand of effluents. A good and reliable method of estimating such rhizosphere oxidation is therefore very important.

The study has also shown that both the intensity and capacity factors of soil reduction may influence some plant growth parameters such as root and shoot weights, and physiological changes, particularly root porosity, which may in turn modulate gas transport in wetland plants. Since CH_4 production appeared to depend partly on root biomass because of provision of extra substrates as exudates and litter, and since root air-space also influences CH_4 oxidation and net emissions, root density and distribution would be essential factors to be considered in any mitigation measures to reduce the CH_4 emissions from rice paddies. Varietal adaptation to increase root density and distribution with correspondingly high porosity should be given an attention in any breeding program aimed at reducing CH_4 fluxes from paddy fields. A combination of good water management in paddy fields to reduce the intensity of anaerobiosis, low organic matter application rates and the use of rice cultivars capable of efficiently oxidizing the rhizosphere would be ideal in the strategy to reduce increases in annual CH_4 emissions.

APPENDIX: LETTERS OF PERMISSION



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VITA

Hillarius Kodzo Kludze was born on September 21, 1954, at Hohoe in the Volta Region of Ghana. He graduated from the University of Science & Technology, Kumasi, in May, 1979, with a B.Sc. (2nd Upper Class Honours) in Agriculture.

He began his professional carrier in 1979 as a researcher in a corn breeding program jointly financed by CIMMYT (Mexico) and the Crops Research Institute of Ghana. Between 1981 and 1985, he was a teacher of Agricultural and Integrated Sciences in Nigeria.. In 1987, he was appointed a Project Co-ordinator/Research Officer for the Volta Region Agricultural Development Project (VORADEP), co-sponsored by the Government of Ghana and the World Bank. Under this project, he conducted on-farm agronomic research trials on legume and grain crops and liased with Ghanaian Research Institutes and Universities affiliated to the project.

He received his M.Sc. (Distinction) in Soil Science in 1989 from the State University of Ghent, Belgium, under the Belgian Overseas Development Fellowship Program. In 1990, he was awarded the Louisiana Methodist Church World Hunger Scholarship to pursue graduate studies in Agronomy at the Louisiana State University. He is at present a candidate for the Doctor of Philosophy degree in Agronomy.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

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Major Field: Agronomy

Title of Dissertation: Gaseous Exchange and Wetland Plant Response to Soil Redox Conditions

Approved:

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Date of Examination:

December 9, 1993